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MALARIA

BASIC PRINCIPLES BRIEFLY STATED

BY

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PREFACE

THE purpose of this monograph is to present the principles of malariology in practical and readable form in a few score pages—perhaps an impossible task. There is not space for references and much material which the author would like to include Readers who require more details may find them in the following publications

- Bates—*The Natural History of Mosquitoes* Macmillan 1949
- Boyd—*Malariology* 2 vols. By 65 authors. W B Saunders Co 1949
- Herms and Gray—*Mosquito Control* The Commonwealth Fund, 1944.
- Macgraith—*Pathological Processes in Malaria and Blackwater Fever* Charles C Thomas 1948
- Russell, West, Manwell—*Practical Malariology* W B Saunders Co 1946

For indexed and classified abstracts of papers and reports on malaria from all over the world, the *Tropical Diseases Bulletin* has been unsurpassed for some forty years It is published by the Bureau of Hygiene and Tropical Diseases in London Another useful periodical is the *Review of Applied Entomology* Series B published by the Commonwealth Institute of Entomology in London Attention is also called to the flow of excellent malaria papers in the *Transactions of the Royal Society of Tropical Medicine and Hygiene* the journals of the National Malaria Society and the American Society of Tropical Medicine also in the *Indian Journal of Malariology* *Documenta Neerlandica et Indonesica de Morbis Tropicis* *Rivista di Parassitologia* the *Rivista di Malariologia* *Mosquito News* *Bulletin of Entomological Research* *Annals of Tropical Medicine and Parasitology* and the *Journal of Economic Entomology* The section on insecticides found in the monthly trade periodical called *Soap* is frequently useful. For more than thirty years the annual *Proceedings of the New Jersey Mosquito Extermination Association* has published helpful material on the control of mosquitoes. Numerous official agencies, such as the Communicable Disease Centre of the U S Public Health Service Atlanta, Georgia the Medical Research

Council (U K.) the Pan-American Sanitary Bureau, Washington and the World Health Organization Geneva, issue malaria bulletins from time to time.

Malaria is an uncommon disease now in the United Kingdom United States and Italy Undoubtedly this enemy of human welfare is retreating dramatically so in some sectors. Yet it continues to prevail in many countries and to cause hundreds of millions to be ill and millions to die each year An anaemia-producing malady it blights entire regions with serious mental apathy and physical deterioration affecting masses of people, so that ambition and working efficiency are blunted. Thus industrial and agricultural development is retarded and the production of food and machines is limited. Malaria undoubtedly is a direct factor in food shortages in some areas.

Then too malaria is an expense to those who live in non-malarious areas Imports from malarious countries carry a malaria tax, probably of not less than 5 per cent, due to the fact that malaria among labourers always increases the cost of what these workers are trying to manufacture or produce. This assessment may total over $175 000 000 yearly for the United States alone. Moreover malaria, wherever it is highly endemic, lowers economic levels so that such areas cannot import as much as otherwise from non-malarious areas.

All in all, malaria still ranks high in the list of man s afflictions. It is dangerous to assume, as too many do that DDT has solved the problem, making it unnecessary for scientists to pay more attention to it, or for sanitary engineers to have further concern about adjusting the environment to prevent anopheline mosquito breeding or for administrators to budget funds for malaria studies. Let us give thanks for modern insecticides and therapeutic drugs but at the same time understand that *they are not panaceas. House flies and* certain *Aedes* mosquitoes are becoming increasingly resistant to economic poisons Perhaps *Anopheles* will follow suit. There is great need for continued entomological and chemical research in laboratory and field to develop cheaper safer and more effective mosquito insecticides and malaria remedies.

Malaria remains a crippling reality to vast numbers of people in the warmer lands. Nevertheless, it is to-day within the power of governments of affected countries to attain a degree of malaria control, even of malaria eradication economically impossible a few years ago Moreover basic understanding and techniques have

progressed so far and the possibilities of laboratory handling are so excellent in malaria that here is a useful tool with which to pry into the secrets of all insect borne disease.

So if the following pages provide orientation as to the present status of malariology they may have usefulness.

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Rome Italy
January 1952

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P F RUSSELL

Rome, Italy

January 195–

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MALARIA

INTRODUCTION

Definition Malaria is an acute and often chronic disease caused by parasites of the genus *Plasmodium* (class *Sporozoa*) and characterized by intermittent febrile paroxysms secondary anaemia, and splenic enlargement. Four species of *Plasmodium* are known to infect man naturally and they result in four kinds of malarial fevers called, respectively vivax malaria, falciparum malaria quartan (or malariae) malaria, and ovale malaria. Mixed infections are not uncommon Obsolete and sometimes confusing synonyms are as follows

Vivax malaria benign, simple, or tertian malaria.
Falciparum malaria estivo-autumnal, malignant tertian pernicious, quotidian subtertian or tropical malaria.
Quartan (or malariae) malaria quartan ague.
Ovale malaria ovale tertian malaria.

Malaria plasmodia require two hosts to complete their life cycle.

The *definitive host* of those infecting humans is always a mosquito of the *Anopheles* genus and the *intermediate host* is man.

History

Prehistoric man was probably subject to malaria. Fossil mosquitoes have been found and fossilized evidence of parasitism exists, dating back to the Paleozoic period. Although these findings do not include anopheline mosquitoes or plasmodia, there seems no reason to doubt that malaria existed in the warmer regions of the earth, at least in Asia and Africa, long before history was recorded. Whether this is so or not, there are undoubted references to characteristically periodic intermittent fevers in early Chaldean Chinese, and Indian medical texts indicating that malaria antedated the Christian era.

In ancient days man apparently had not the faintest inkling of the aetiology of malaria, nor had he found any effective treatment. The dramatic febrile paroxysms which came with so little premonition were due to the caprice or sly design of angry gods, or were

punishment sent by stern dieties for wrongdoing or were the work of malevolent spirits—unpredictable supernatural beings to be deceived by artifice placated by incantation or propitiated by sacrifice.

Malaria appears to have become endemic in Greece by 400 B C. and may have been a factor in the decline of physical vigour intellectual power and moral fibre so clearly evident a century later Greek physicians differentiated quotidian, tertian, and quartan fevers, and even non-medical writers like Plato used these terms. In Rome, references to such fevers date from about 200 B C. and came from the pens of writers like Plautus and Terence, Cicero Celsus, and Pliny the Elder Cicero attributed the regular periodicity to the will of the gods But many physicians by that time, in the East as well as in Greece and Rome, had come to believe in natural causation of disease a tremendous forward step in the progress of medical science. To be sure, natural causes included assorted febrific airs, lunar rays, and terrestrial perturbations, but there was obvious searching for truth. Both Greeks and Romans suggested an etiologic relationship between marshes and the intermittents. Both began to discuss season and terrain in reference to these fevers, and both attempted preventive drainage.



Fig 1 An ancient Roman drainage pipe

Malaria may not have occurred in the Americas prior to Columbus. Historians do not agree, but some believe that it first appeared at Isabella on the north shore of Hispaniola in 1493 as an epidemic fever among the colonists. Human carriers and anopheline vectors spread the disease throughout tropical America and even as far north as Canada. Australia may also have been free from malaria until Europeans carried it there. At any rate, by the seventeenth century the intermittent fevers, agues, or Roman airs were notorious the world over

There were no advances in aetiology epidemiology or prevention of malaria for some sixteen centuries. But about 1640 came the beginning of modern malaria therapy when cinchona bark was taken from

Peru to Europe and crude powdered bark became the sovereign remedy for paroxysmal fever After two French pharmacists Pelletier and Caventou isolated quinine and other alkaloids from the bark in 18_o the standard drug for malaria was quinine which was commercially available in the United States as early as 18 3 and was not displaced as the remedy of choice for over 100 years. The use of bark enabled Morton and Sydenham in England in 1666 and specially Torti in Italy in 171_ to differentiate those fevers alleviated by cinchona powders from those unaffected thus sharpening the focus on the intermittent fevers now called malarial.

During the eighteenth century malaria got its name Italians had formed the habit of saying that patients who died of an intermittent fever had been victims of bad air or *mal'aria* Horace Walpole in 1740 referred to the disease as a horrid thing called *mal' aria* that comes to Rome every summer and kills one Thus arose the expression malarial fevers and finally the modern word *malaria* which now means not an aetiologic agent but a group of diseases.

The nineteenth century was one of classic discoveries in bacteriology and pathology Raimbert in 1869 showed experimentally that anthrax could be disseminated by flies. Snow and Budd by their studies of the epidemiology of cholera and typhoid fever Pasteur and Koch by their brilliant laboratory findings, paved the way for Laveran the French Army surgeon who in 1880 in Constantine Algeria, was first to see and to describe malaria plasmodia as parasites in man s red blood cells.

During this same period occurred (1) the inspired theorizing of Patrick Manson in 1877–84, on the basis of his original observation of a mosquito acting as an intermediate host to a filarial parasite of man (2) the proof by Theobald Smith and Kilborne in 1889–93 that a specific pathogen *Babesia bigemina* could be transmitted from one animal to another by invertebrate tick hosts (*Boophilus annulatus*) (3) M. B Waite s discovery in 1891 that bees and wasps are vectors of fire blight, a bacterial disease of pears and other fruits (4) the demonstration by David Bruce in 1896 that a disease *nagana* caused by a protozoan parasite could be transmitted from animal to animal by a true insect (tsetse fly) and finally (5) William George MacCallum s studies of exflagellation in 1897–98 which indicated that there was a sexual phase in the development of malaria parasites.

TABLE I

Some Achievements in Malaria History During the Twentieth Century

Achievements	By	Dates
First large-scale demonstrations of malaria control through mosquito abatement.	Gorgas in Cuba and Panama Canal Zone. Watson in Malaya.	1899–1914 1901–27
Demonstration of first economically feasible malaria control in rural tropics through pyrethrum spray killing of adult anophelines.	Numerous workers in South Africa, Netherlands, and India.	1935–39
Synthesis of isomers of cinerin I.	La Forge and colleagues.	1947–49
First demonstrations of the eradication of an invading malaria vector—*A. albimanus* in Barbados and *A. gambiae* in Brazil and Egypt.	Barbados Government. Brazilian and Egyptian Governments, co-operating with the International Health Division of the Rockefeller Foundation under leadership of Soper and Wilson.	1927 Brazil 1939–40 Egypt 1943–45
First effective antimalaria protection of large military forces in highly endemic areas.	Specially trained, full-time malaria survey and control personnel of the Allied Forces in World War II	1940–45
Initiation of first projects by governmental agencies aiming at complete eradication of malaria from entire countries.	Venezuela. Italy Cyprus. U.S.A. Mauritius.	1945 1946 1946 1947 1948
Development of such insecticides as DDT lindane, and chlordane, and of the residual-spraying technique against adult anophelines.	Synthesis of DDT by Zeidler Discovery of insecticidal properties of DDT by Paul Müller and Robert Wiesmann. Further development and mass production by U.S.A. and U.K. through numerous governmental and private agencies.	1874 1936–39 1942 to date
Synthesis and development of plasmochin.	Schulemann and colleagues.	1924–27
Synthesis and development of atabrine	Mauss, Mietzsch, Kikuth, and colleagues.	1930–33
Development of several 4- and 8-aminoquinolines as useful therapeutic and suppressive agents in malaria.	Many workers in Germany and the U.S.A. under both governmental and private auspices.	1940 to date
Synthesis and development of chlorguanide.	Many workers in the U.K. under both governmental and private auspices.	1940 to date
The beginnings of elucidation of the exoerythrocytic stages of malaria plasmodia	Avian malaria—first by Raffaele, then by Huff and colleagues, James, Mudrow and others. Simian malaria—Shortt, Garnham Malamos, and others. Human malaria—Shortt, Garnham, Covell, and colleagues.	1934 to date 1948 to date 1948 to date

ents preceded the discovery by Ronald Ross in 1897–98 that of man and birds is a mosquito-borne disease. The Italians, Bignami, G Bastianelli, and Battista Grassi quickly proved hat human malaria is transmitted by one genus of mosquito *nopheles*

notable peaks in the subsequent history of malaria during half of the twentieth century are outlined in Table I

Classification The malaria parasites of man, monkey bird, and other vertebrates are all protozoa of the class *Sporozoa* and of the genus *Plasmodium* described by Marchiafava and Celli in 1885 There are four recognized species in man as follows

Plasmodium malariae (Laveran) 1881
Plasmodium vivax (Grassi and Feletti) 1890
Plasmodium falciparum Welch 1897
Plasmodium ovale Stephens, 1922

Life history So far as is known, all plasmodia of man and animals spend a part of their life in vertebrate and part in mosquito hosts Some observers have suggested that certain plasmodia of lizards and bats may have arthropod hosts other than mosquitoes, but this is still doubtful.

Malaria parasites have four phases of development (1) the *sexual phase* starting with the growth of gametocytes in the vertebrate host and continuing with sporogony in the tissue of the mosquito (2) the *pre-erythrocytic development* from sporozoites (3) *asexual schizogony* in the red blood cells (4) *exoerythrocytic schizogony* The second and fourth stages in mammalian malaria are still in the shadows.

Development in Mosquito

Sexual phase The sexual stages of development of plasmodia start with the formation of male and female *gametocytes* in the vertebrate host, as described below When these are ingested by the mosquito development continues within the insect's body as a process of *sporogony* leading to the production of *sporozoites* which in turn infect the vertebrate host.

When mature gametocytes are taken into the gut of a susceptible mosquito there is *gametogenesis* in the lumen of the insect s stomach. In this process the female gametocytes become rounded *macrogametes* and each male gametocyte by *exflagellation* forms eight to ten thread like *microgametes* which separate, each attempting to fuse with a macrogamete in a process of fertilization. A fertilized cell, within 12 hours after the infective blood meal, at 26° C (78 8° F) becomes an elongated mobile *zygote* better called *oökinete* or *vermicule*. This

migrates through the stomach wall of the insect, coming to rest between the epithelial lining and the elastic outer membrane of the stomach where it develops into an *oocyst* The youngest recognizable oocysts appearing in about 4 days measure a micron or two in diameter but those fully grown may be 50 to 60 microns.

Within the oöcyst hundreds of chromatin-bearing spindle-shaped flecks of cytoplasm develop and when these fusiform *sporozoites* are mature, the oocyst bursts. The sporozoites, each some 8 to 12 microns long travel in the haemolymph of the insect's body cavity to the salivary glands into which they penetrate They remain in salivary cells and free in the salivary ducts. Thus whenever the mosquito injects saliva into a victim sporozoites also are injected

Asexual Development in Vertebrate Host

Exoerythrocytic schizogony Formerly it was presumed that after entering the blood stream of a non-immune the sporozoite would penetrate a red blood cell to initiate the erythrocytic cycle described below It is now known that, in at least five species of avian and one of saurian plasmodia, there are intervening *exoerythrocytic* stages during which the parasite is living in cells other than erythrocytes and reticulocytes. The nature of the exoerythrocytic forms and development of mammalian plasmodia remains somewhat doubtful. Such stages would explain the cryptic phase in sporozoite-induced vivax and falciparum infections—a phase during which pre-erythrocytic development may be proceeding in some situation other than the blood stream. Sporozoites circulate in the blood for about half an hour after inoculation and then they disappear After this the blood is not infectious until towards the end of the incubation period.

In vivax and falciparum infections, no stages of the parasite have ever been detected during the first 3 days. It is not known whether the sporozoites directly enter liver parenchyma cells, where some observers believe they have found pre-erythrocytic schizonts from the fourth day or whether they directly enter both parenchymatous and Kupffer cells or whether they undergo their earliest changes in reticulo-endothelial cells the resulting stages entering liver parenchyma cells Other kinds of sporozoites such as those of the avian *P gallinaceum* enter cells of the lymphoid-macrophage system of the skin or as has been suggested for *P elongatum* prefer cells of the erythroblastic series. There are species differences and still other types of fixed tissue cells may be involved (The term

protozoite has been applied by French authors to forms said to
from the primary division of sporozoites)

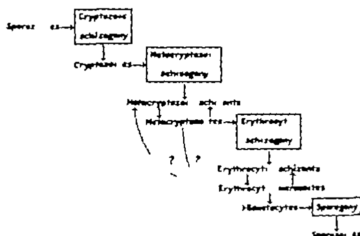


Fig. 2. Probable Chain of Events i Life of *Plasmodium*
There are species differences. Moreover the exact happenings in the case of man
plasmodia ha e not been conclusively demonstrated.

On the basis of observations in avian malaria, it appear
within the first vertebrate host cell there takes place *cryp
schizogony* with the production of *cryptozoites* or *cryptozoic meroz*
or *cryptozoic schizonts* arising directly from sporozoites. Cr
zoites on liberation, may enter other fixed tissue or non-erythro
cells initiating further schizogony The progeny of the secon
of succeeding generations of *pre-erythrocytic schizogony* are
metacryptozoites Some of the metacryptozoites specially
three or four generations, appear to penetrate erythrocyt
initiate *erythrocytic schizogony* described below Following
appearance of erythrocytic forms it is illogical to apply the ex
sion pre-erythrocytic' to newly-formed parasites. The
phanerozoite and *phanerozoic stages* are used to name all exoery
cytic forms of the parasite appearing concomitantly with or
sequent to erythrocytic parasites. These terms are also use
exoerythrocytic stages seen in blood-induced infections in w
the sporozoites do not take part.

Certain exoerythrocytic schizonts have many nuclei but s
cytoplasm, and have been called *microschizonts* They produce
numbers of *micromerozoites* which resemble merozoites. Ther
also produced *macromerozoites* in smaller numbers and of larger
It has been suggested that the former enter red cells and the l
tissue cells.

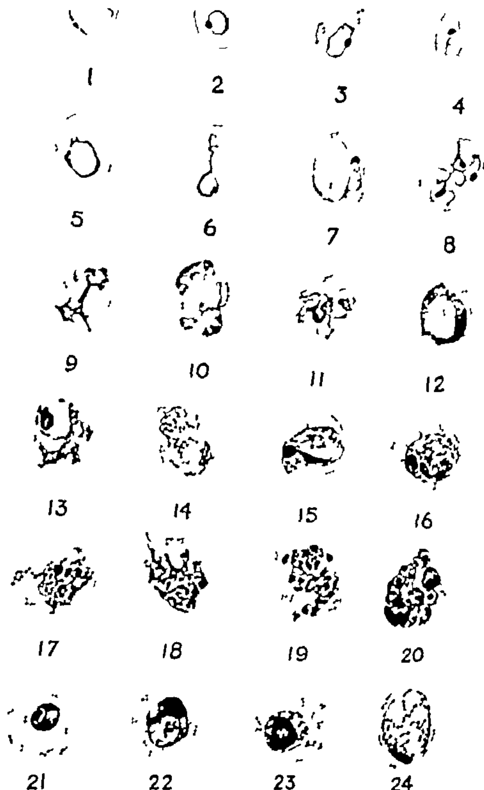


FIG. 3. *Plasmodium vivax* Thin film appearance, Giemsa stain. (Courtesy National Institutes of Health, USPHS.) 1 Normal size red cell with marginal early trophozoite. 2, Young signet ring form in a macrocyte 3 Slightly older stage in red cell showing basophilic stippling. 4 Polychromatophilic red cell with early trophozoite. 5, Trophozoite with pigment in red cell showing Schüffner dots. 6, 7 Tenuous medium age trophozoites. 8, Cell containing three ring forms which appear fused. 9, 11 12, 13, Older trophozoites showing irregular cytoplasmic scatter. 10 A doubly infected erythrocyte. 14, Mature trophozoite. 15, Mature trophozoite with chromatin beginning to divide. 16, 17 18, 19, Presegmenters. 20, Segmenter 21 22, Developing gametocytes. 23 Microgametocyte 24 Macrogametocyte.

facing p. 9

Thus it appears that there is a line of development from sporozoite to cryptozoite to metacryptozoite to erythrocytic stages and the terms themselves probably designate rather loose categories merging from one into the next. Moreover in some species not only do cryptozoites, metacryptozoites, and phanerozoites penetrate fixed tissue or other cells to initiate new exoerythrocytic schizogony but perhaps some merozoites from the erythrocytes also go into fixed tissue cells, instead of only entering other erythrocytes as formerly supposed. This point is not clear but possibly exoerythrocytic schizogony may be initiated not only by sporozoite, cryptozoite metacryptozoite or phanerozoite, but also by a merozoite developed in an erythrocyte This may conceivably be the case with *P vivax* but perhaps not with *P falciparum* for it may be that complete eradication of erythrocytic infections in falciparum malaria ends the disease and relapses do not thereafter occur

Erythrocytic schizogony When first seen in (or upon) the erythrocytes, the malaria plasmodium appears as a minute speck of chromatin surrounded by scanty cytoplasm. It gradually becomes ring-shaped and is called a *ring* or a *trophozoite* Most observers believe that the erythrocytic plasmodium is intracellular but some consider that it may be attached to the outside of the red cell at least part of the time As trophozoites develop some species, such as *P vivax* are quite ameboid but all tend to become more evenly rounded and solid. When the chromatin is about to divide the trophozoite becomes a *schizont* In strict protozoological terminology all asexual forms with multiple division of nuclei are *schizonts* and the earlier undivided asexual parasites are *trophozoites* After division begins schizonts are called *presegmenters* until the chromatin has fully divided and the *merozoites* have taken shape. When this has occurred, the parasite is called a *mature schizont* or *rosette* or *segmenter* The term *cytomere* is sometimes used to describe those intermediate masses or bodies of a schizont which actually produce the merozoites. Schizogony is fulfilled when the merozoites have completely separated and the host erythrocyte has disintegrated, scattering the merozoites. Most of these enter new red cells but some may penetrate fixed tissue cells, as noted above.

This process of *asexual multiplication* may continue for days, weeks or even years Often there is in this asexual cycle a characteristic periodicity that becomes manifest in clinical quotidian, tertian, or quartan paroxysms. These occur when the blood plasma is flooded

with merozoites, malaria pigment, and red cell debris at the time of *sporulation* or *schizogony*

Some of the merozoites which enter red blood cells do not undergo schizogony but develop within the erythrocytes into sexual *gametocytes* The males are called *microgametocytes* the females are *macrogametocytes* and are more numerous than the former Gametocytes reach maturity but do not achieve their destiny in the vertebrate host. If not ingested by the insect, these gametocytes are phagocytosed and destroyed within a few days.

Physiology of plasmodia The malaria plasmodia are obligatory intracellular parasites about which actually very little is known, apart from morphology and life cycle. Recently in a few laboratories attempts have been made to obtain a better understanding of the metabolism of the parasite, specially with reference to a more rational approach to culture *in vitro* and to chemotherapy Respiration various cellular oxidative processes, enzymes, and co-enzymes are under investigation. Such studies indicate, for example, that malaria parasites utilize oxygen by means of a heavy metal respiratory enzyme, possibly the iron porphyrin cytochrome oxidase It is not known whether or not malaria plasmodia can long survive complete anaerobiosis. Other observations suggest that it is the need for globin which causes plasmodia to split haemoglobin into haeme and globin. The haeme becomes haemozoin which is malaria pigment. The exoerythrocytic stages in non-haemoglobin containing cells produce no pigment. The protein of the red cells is broken down by hydrolysis or phosphorylysis and about half of the amino acids are utilized by the plasmodia for the synthesis of their own proteins, while the remainder diffuse out of the erythrocyte.

Diagnosis

Tables II–IV list thin and thick stained film characteristics and some comparative developmental periods of the four species which parasitize man.

Preparation of blood smears For malaria blood smears, use new 3 × 1 glass slides which have been thoroughly dried and polished after having been put through an alkaline wash and alcohol rinse. Some of the new detergents such as triton, may clean better than an alkaline wash but thorough rinsing is necessary before the final alcohol rinse. If old slides are used, they require a bichromate wash and alcohol rinse and they should be free from defects or scratches. Blood for smears may be obtained by pricking ear lobe,

finger or toe In most cases the ear lobe seems best but among people who use quantities of hair oil the ball or perhaps the back of a convenient finger is preferable, and when working with infants the big toe is best. In any case, the site is thoroughly cleaned with alcohol-soaked cotton and when dry is punctured with a suitable lancet, Hagedorn needle, or half pen-point.

Thin and thick smears may well be taken on the same slide. To make the thin smear touch the surface of the slide to a small fresh drop of blood, at a point one third distant from one end With this slide evenly supported as on a table, touch the drop with the lower end of another clean slide By capillarity the drop will spread along the edge of the second slide The latter is held at an angle of 30 to 45 and gently but rapidly pushed along the first slide to spread the blood into an even thin smear After cleaning the puncture site a large drop of fresh blood is allowed to touch the middle of the unused third of the slide carrying the thin smear The blood adhering to the glass is then briefly puddled with a corner of the spreader to form a thick smear about 1 cm. in diameter and 50 μ or less thick. It is not necessary to difibrinate the thick drop Brief data should be written with a hard-lead pencil on the thin smear itself. The smears must be protected from such hazards as dust, ants, cockroaches and flies. In very humid environments a fan or hair-dryer may be used to prevent injury to films by absorption of moisture during slow drying Plastic slide boxes may delay drying to the detriment of the smears. Essentials to be emphasized are clean slides, clean skin where pricked, protection of smear from dust, moisture and insects and finally a good stain.

Giemsa's Stain 1 Fix thin but *not* thick smear in absolute methyl alcohol for 30 seconds. The thick film must not come into contact with the alcohol

2. Transfer slide to staining solution which should be freshly prepared by mixing enough stock solution with buffered water (pH 7·2) to make a dilution of one to fifty Stain both thick and thin smears simultaneously for 45 minutes.

3 Wash with tap water and stand upright to dry

To prepare Giemsa's stock solution take 1·0 gm. powdered stain (Giemsa stain Azure B type for malaria and blood work as certified by the Biological Stain Commission) 66·0 c c glycerin (C.P) and 66·0 c.c. methyl alcohol (absolute acetone-free) Grind powdered stain and glycerin together When well mixed dissolve

TABLE II

Comparative Characters of Plasmodia of Man

Stained thin smears

Stage	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium falciparum*	*Plasmodium ovale*
Early Trophozoite or Ring	Relatively large usually one chromatin dot, but sometimes two, often two rings and sometimes more in one red cell.	Compact one chromatin dot rarely double infections.	Small, delicate in early stages, later fleshy and pigmented sometimes two chromatin dots multiple infection of red cells common appliqué forms frequent.	Compact one chromatin dot double infection uncommon.
Late Trophozoite	Large markedly amoeboid prominent vacuole pigment, fine rodlets.	Small often band-shaped not amoeboid vacuole inconspicuous pigment coarse.	Medium size usually compact, rarely amoeboid vacuole inconspicuous rare in peripheral blood after half-grown pigment granular	Small compact not amoeboid vacuole inconspicuous pigment coarse.
Young Schizont or Presegmenter	Large somewhat amoeboid chromatin masses numerous pigment fine rodlets.	Small, compact chromatin masses few pigment coarse.	Medium size compact chromatin masses numerous pigment granular rare in peripheral blood.	Medium size compact chromatin masses few pigment coarse.
Mature Schizont or Segmenter	Larger than normal b.c. may have double rosette.	Smaller than normal b.c. single rosette.	Smaller than normal b.c. irregular rosette rare in peripheral blood.	Larger than *malariae* irregular rosette.
Number of merozoites	8 to 16, usually 12 15	6 to 12, usually 8.	12 to 24 (or more) but usually 8–12.	6 to 12, usually 8.

Macrogametocytes (usually smaller and less numerous than macrogametocytes)	Spherical compact no vacuole single large nucleus diffuse coarse pigment cytoplasm stains light blue	S mila to ri ax but smaller and less numerous	Crescents usually sausage shaped hromatin d ffuse pigment scattered rice grains nucleus rather large cytoplasm stains darker blue	smaller never abundant.
Macrogametocytes	Spherical compact larger than microgametocyte sm l- ler nucleus pigment same cytoplasm stains darker blue.	Similar to vivax but smaller and less numerous	Crescents often longer and more slender hromatin central, pigment more compact nucleus compact cytoplasm stains darker blue.	Similar to vivax but somewhat smaller never bundant.
Pigment	Short, rather delicate rodlets irregularly scattered not much tendency to coalesce	Seen in ery young rings granules rather than rods tendency toward peripheral scatter	Pigment granular early tendency t coalesce typical single solid mass in mature trophozoite coarse scattered rice grains in crescents.	Similar but somewhat coarser than vivax
Alterations in the infected erythrocyte	Enlarged and decolourized Schuffner dots usually seen.	May appear smaller fine stippling (Ziemann dots) occasionally seen	Normal size, but may ppear "brassy Maurer dots (or clefts") may be seen host cell of crescent barely discernible.	Enlarged and decolourized Schüffner dots ppear early and are prominent at all stages numerous val-shaped red cells, or with crenated margins.

Thick Film Characters

Plasmodium vivax malariae and falciparum

Stage	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium falciparum*
Early Trophozoite	Fairly numerous irregular cytoplasm fairly large single chromatin bead often mixed with later stages.	Few more regular cytoplasm medium size single chromatin bead may be segmenters present.	Often very numerous delicate cytoplasm small, sometimes double chromatin bead no other forms usually present except perhaps crescents.
Half-grown Trophozoite	Great irregularity of cytoplasm which tends to scatter away from single chromatin blob few small granules of pigment.	Regular compact cytoplasm contracting around single chromatin bead pigment forms early and tends to concentrate.	Not common in peripheral blood regular cytoplasmic ring, broken ring, and comma patterns single or double chromatin bead.
Late Trophozoite	Considerable cytoplasmic scatter and irregularity chromatin blob often isolated fine granular pigment with moderate dispersion and perhaps isolated from cytoplasm, other stages usually present Schüffner dots sometimes seen.	Numbers generally few older stages present rounded, compact cytoplasm often obscuring chromatin scattered pigment, relatively bundant.	Not in peripheral blood except in very heavy infections solid, irregularly rounded chromatin indistinct pigment concentrated.
Early Schizont or Presegmenter	Large amount of cytoplasm loosely covering bundant chromatin which is beginning to segment pigment granules discrete and lightly concentrated in one or two areas.	Smaller and not so numerous some scatter of cytoplasm and segmentation of chromatin pigment in small separate granules.	Generally not in peripheral blood but if so will be associated with numerous typical ring forms irregular fairly compact, dark staining pigment fused in single mass.
Mature Schizont or Segmenter	8–16, usually 12–15 merozoites elatively large size early vacuole formation pigment granular and clumped other stages often present.	6–1., usually 8 merozoites, each with vivid purple, ovoid bead of chromatin early vacuole formation pigment compact clump of discrete granules light infection, smaller size	Rare in peripheral blood 12–24 or more merozoites fairly uniform ovoid or round chromatin beads merozoites grouped or scattered pigment a single dark mass.
Gametocyte	Round or oval, relatively large with fairly uniform cytoplasm somewhat frayed at edges small rodlet-shaped pigment, irregularly scattered abundant chromatin, more diffuse in males.	Rounded, compact, with bundant peripheral pigmentation in round granules single chromatin mass ften bscured and more diffuse in males.	When mature and normal has distinctive crescentic shape females longer and more slender with central pigment and chrom tin males fatter and paler with scattered pigment and diffuse chromatin; coarse rice grain pigment.

TABLE IV

Comparative Developmental Periods of Plasmodia in Man

Period	*P. vivax*	*P. malariae*	*P. falciparum*	*P. ovale*
Length of asexual phase	48 hours (or a little less)	72 hours	48 hours	48 hours
Prepatent period in man (minimal)	8 days A 13–17	17 days Ave 28–37	5 days A c 8–12	8 days
Usual incubation period in man	8–31 days A 14	28–37 days A 30	7 27 days Ave 1	11 16 days Ave 14
Gametocytes appear following parasite patency (gametocytes generally infectious few days after appearance)	3–5 days	7 14 days Appearance irregular and numbers few	7 12 days	12 14 days Appearance irregular and numbers few
Developmental period in mosquito	16–17 days at 20° C. (68° F.) 10 days at 25 C. (77° F.)	30–35 days at 20° C.	22 23 days at 20° C	16 days at 25 C.

stain in glycerin in a waterbath at 55–60 C When cool add methyl alcohol allow to stand for 2 or 3 weeks then filter for use

Prepared stock solutions can be purchased.

Jaswant Singh and L. M Bhattacharji or J S.B Stain (1) Fix thin but not thick film in absolute methyl alcohol for 30 seconds. (2) Dry by waving slide in air (3) Immerse thick and thin slides in J S.B solution I for 30 seconds. (4) Wash in acidulated tap water (pH 6·2–6·6) (5) Stain in J S.B solution II for 1 second. (6) Repeat step § 4 for 4 seconds. (7) Repeat step § 3 (8) Repeat step § 4 for 10 seconds or until thin smear has a pink background. (9) Stand upright to dry

If thick smears alone are being stained the process may be shortened as follows (1) Immerse in J S.B solution I for 10 seconds. (2) Wash in acidulated water (pH 6·2–6·6) 2 seconds. (3) Stain in J S.B solution II for 1 second (4) Repeat step § 2 for 5 seconds. (5) Repeat step § 1 (6) Repeat step § 2 until smear shows pink background. (7) Stand upright to dry

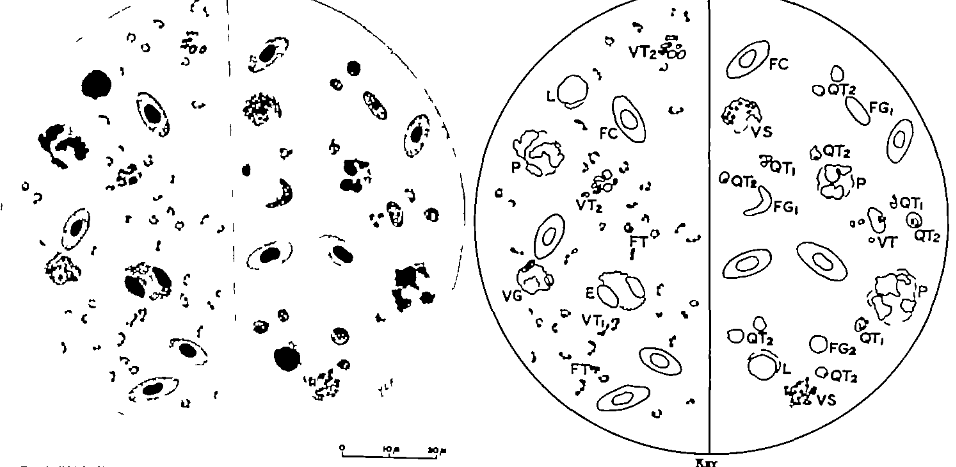


FIG. 6. Thick films made with equal quantity of an avian red cell suspension of known cell concentration to facilitate parasite counting. (From J W Field, *The Microscopic Diagnosis of Human Malaria.* Studies from the Institute for Medical Research, Federation of Malaya. No. 23, 1948.)

Key to Fig 6 FC, avian erythrocytes. *P* polymorph. *L*, lymphocyte. *E*, eosinophil. *FT* *falciparum* early trophozoites, distinctive size and numbers. *FG* *falciparum* characteristic gametocyte FG_2, contracted *falciparum* gametocyte resembling late trophozoite or gametocyte of malariae *VT* *vivax* early trophozoites, not distinctive. *VT* VT_2, late *vivax* trophozoites with characteristic cytoplasmic scatter *VS* typical *vivax* segmenter *VG*, *vivax* gametocyte with characteristic size, shape and undivided chromatin. *QT* early *malariae* trophozoite, not distinctive QT_2, late *malariae* trophozoite with distinctive small, compact form.

J.S.B. solution I is prepared from th[illegible]

Medicinal methylene blu[illegible]	[illegible]
Potassium dichroma[illegible]	[illegible]
Sulphuric acid (1 per cent by volum[illegible])	[illegible]
Water	[illegible]

Dissolve methylene blu[illegible] m[illegible] add [illegible] well and then add th[illegible] dichromate. H[illegible]at [illegible] 109 C. (not over 110 C.) at [illegible] still greenish, heat for an[illegible] her hour or [illegible] temperature. Then whil[illegible] 10 c.c. of 1 per cent KOH or NaOH. [illegible] flasks and shake each for 15 min[illegible] precipitate has di[illegible]solved and [illegible] violet irridescence. Stand 48 hours [illegible] through soft filter paper and th[illegible] it improves with age.

J.S.B. solution II is prepared [illegible] 500 ml. of tap water. After th[illegible] gives better results than imm[illegible]

In all mass staining w[illegible] washing of para[illegible]ite material fr[illegible] small quantity of a [illegible]urface activ[illegible] water seems to help prevent [illegible] the slides are only separate[illegible] occur in the usual staining [illegible]

Examination of blood films. Stan[illegible] thick smears for 5 minutes [illegible] at finding a parasite, but usually [illegible] order to confirm species diagnosi[illegible] if present. While species diagnosi[illegible] made in a thick film it is sometimes useful [illegible] for confirmatory evidence.

The following artifacts in thi[illegible] films may be [illegible] with malaria parasites. (1) *Blood plat[illegible]* individually or in small groups; single platelets may suggest early [illegible] quartan [illegible] platelet clusters may resemble m[illegible]re mature [illegible] in which the chromatin is intact but the cytoplasm somewhat fragmented; (2) denucleated debris from immature erythrocytes and tags of red-cell

reticulum common in anaemic blood and correspond to the Howell-Jolly bodies of thin films sometimes when in chance

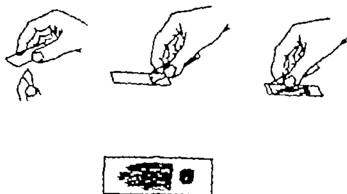


FIG. 7 Diagram of manner of making thin and thick blood smears.

relation to blue-staining material, they look like young trophozoites (3) *immature red-cell reticulum* stained deeply enough to resemble *vivax* trophozoites surrounded by thick film equivalent of Schüffner's dots (4) *adventitious structures* (not found in clean, well-stained smears) include dust, moulds, yeasts, vegetable spores, bacteria, and stain granules.

Diagnostic criteria are given in Tables II and III. A few of these may be emphasized. For instance, erythrocytes infected with *vivax* or *ovale* are enlarged and pale by the time the parasites have grown beyond the early ring stage. *Ovale* host cells may show ovoid distortion with partly fimbriated edge. Differentiation of very young individual rings of *vivax ovale* and *malariae* is rarely certain In properly stained erythrocytes containing *vivax* or *ovale* Schüffner's dots are usually present, earlier and coarser in the case of *ovale* than *vivax* The Maurer's and Ziemann's dots of *falciparum* and *malariae* are much less consistently seen.

Note that in properly stained thick films the early trophozoites of each of the four species always show a bead of dull red chromatin attached or close to a fleck of pale blue-purple cytoplasm. Without these two characters the object seen is not a young malaria parasite. These young trophozoites usually appear as rings, broken rings, gull's wings, swallows, commas and exclamation points. Such patterns are more consistent and persistent in *falciparum* The earliest *falciparum* forms, clinically most important, are so minute that they are easily missed if the smear is dirty or poorly stained.

Species differentiation may not be possible in the earliest stages.

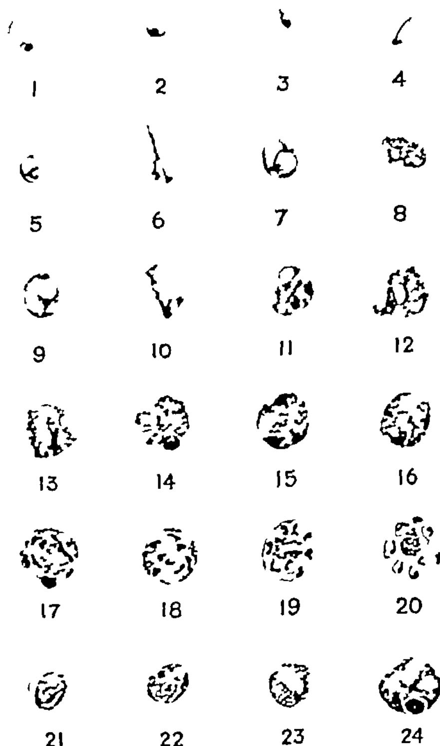


Fig. 4 *Plasmodium malariae* Thin film appearance Giemsa stain. (Courtesy National Institutes of Health, USPHS) 1 2, 3, 4 Young trophozoites. 5 Older trophozoite showing pigment granule 6, Early band form 7 8, 9 10 11 12, Developing trophozoites. 13 14 Mature trophozoites, one band form 15, 16, 17 18, 19 Presegmenters. 20, Segmenter with the usual 8 merozoites 21 22, Young gametocytes. 23, Mature microgametocyte. 24 Mature macrogametocyte

Facing p. 19

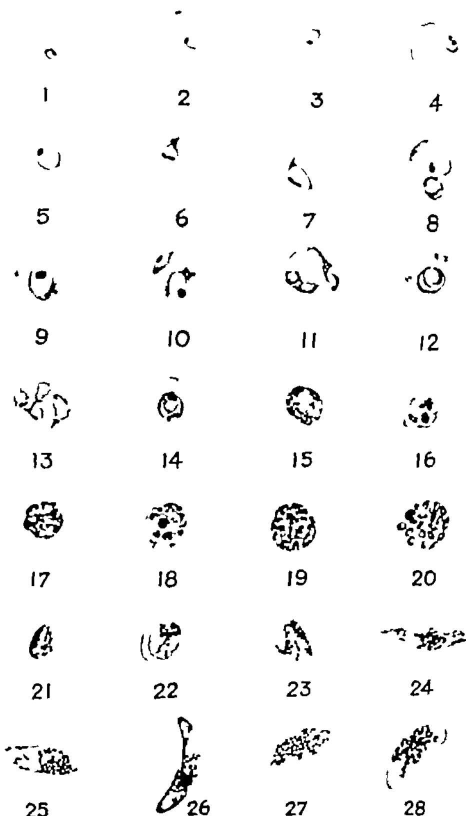


FIG. 5. *Plasmodium falciparum*. Thin film appearance, Giemsa stain. (Courtesy National Institutes of Health, USPHS.) 1 2, 3, 4 5, 6, 7 Young trophozoites. 8, Triple infection in one cell. 9 10, 11 12, Older trophozoites showing pigment and sometimes Maurer's spots. 13 'Slender forms. 14 15 Mature trophozoites. 16, 17 18, 19 Presegmenters. 20, Segmenter 21 22, Young gametocytes (forms 14 to 22 are rare in peripheral blood) 23, 24 25, 27 Immature gametocytes. 26, Mature macrogametocyte. 28, Mature microgametocyte.

freezing in defibrinated or citrated blood, *P vivax* ha viable for many months. There are also methods such Bass and Johns, Hawking and Ball and Geiman for at culture plasmodia there are special techniques for egg plasmodia for malaria tissue smears and for the pre sections of liver spleen, and of marrow punctures or *exceptional cases, sternal puncture may be a useful sup* diagnostic method. Finally passing mention may b numerous diagnostic tests such as melano-flocculation melanin-serum (Trensz) protein-tyrosin (Proske and precipitin and complement-fixation Details of all o others will be found in standard textbooks and all abstracted in the *Tropical Diseases Bulletin* None has y much practical usefulness. Wasserman and Kahn te falsely positive in malaria.

Plasmodia of Animals

There are several well-known plasmodia in monkey *molgi P inui P knowlesi* and *P kochi* for exampl chimpanzees in Central Africa appear to be naturally in the identical *P malariae* found in man. In birds at le species have been studied, including *P gallinaceum, P c* *P relictum P lophurae P elongatum P hexamerium* Several plasmodia have been reported from bats, sq rodents One of the most recent and one which may p in laboratory studies is *P berghei* from a Congo tree infect laboratory rats and mice, but thus far a suitable vector has not been found. There are also plasmodia in in amphibians but these have not had much study

PATHOLOGY CLINICAL COURSE AND THERAPY

Pathology

Pathogenesis Regardless of species of plasmodium involved in human malaria three basic factors determine the pathology of the disease (1) degree of multiplication of parasites and hence the amount of pigment produced (2) degree of erythrocyte destruction by the parasites and perhaps by malarial lytic processes and (3) degree of phagocytosis established by the host's defensive forces. The latter lie for the most part in the reticulo-endothelial system, chiefly of the spleen liver and bone marrow where there is relatively slow movement of blood and an intimate contact between parasite and reticulo-endothelial cell.

The destruction of red blood cells and the blood dilution mentioned below lead to anaemia and so to a generalized anoxaemia, made more severe by the fact that the parasite itself utilizes oxygen from the oxyhaemoglobin of the cell and is also continually splitting haemoglobin into haeme and globin. Moreover in severe cases there may be interference with pulmonary function still further reducing the supply of oxygen to the body The anoxaemia and probably some factor inherent in the malaria host parasite struggle, do injury to vascular endothelium, in the brain and elsewhere, and this damage in turn may result in abnormal permeability The capillaries and precapillaries are distended and sometimes occluded by parasitized cells seriously retarding the blood flow There may be margination of parasitized cells in larger blood vessels due to surface agglutinins and to mechanical factors. This leads to the formation of 'sludge and the blood flow may be so disrupted as to induce shock. The changed blood flow anoxaemia, and endothelial damage may lead to general tissue anoxia, complicated by various immune reactions, by auto-antigens and possibly even by histo-toxic agents At first the tissue changes are reversible—remarkably so But in cases of continued severity sooner or later degeneration and necrosis occur with permanent damage and even death

There may be a circulating diffusible toxin possibly a polypeptide fraction liberated at the time of schizogony when the red cell disintegrates. Some such agent might conceivably affect the

hypothalmic centres and initiate the characteristic malarial paroxysm. But no malaria toxin has ever been identified.

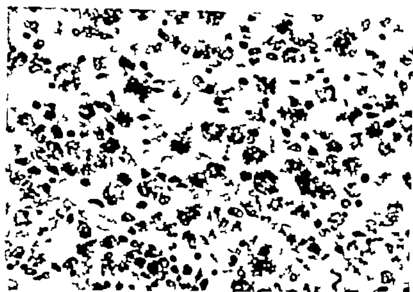


Fig 8 Spleen in falciparum malaria, showing hemozoin in cells. (From Maegraith, *Pathological Processes in Malaria and Blackwater Fever* Charles C Thomas, Publisher after Ash & Spitz, 1946.)

Spleen The spleen is always affected in malaria. The first changes are those of acute congestion but gradually there is an increase in pigment so that the spleen becomes darker Macrophage elements multiply causing the organ to develop into the ague-cake or enlarged, palpable spleen so typical of malaria. Normally the spleen in life probably weighs about 3 8 gm. per kilogram of body weight or say about 276 gm. (11 8 oz.) in a man weighing 160 lb (72·7 kg) In malaria, spleen weight may increase to 700 or 800 gm. (30·0–34 3 oz.) in an acute attack, and to as much as 5 000 gm. (213 1 oz.) in chronic cases.

Microscopically the spleen at first reveals only hyperaemia. But as the disease continues, pigment and parasites in all stages of development become abundant in the pulp not only within parasitized red cells, but lying free as well also crammed into large numbers of giant phagocytic cells. Other usual findings are diffuse cellular hyperplasia, dilated sinuses, and occasional thromboses of capillaries and local foci of toxic necrosis in the splenic pulp In chronic malaria, connective tissue of the spleen may be considerably increased.

Liver The liver is also swollen and darkened in malaria. The

Kupffer cells are enlarged and more numerous, and are usually pigmented. Capillaries may be distended with macrophages



FIG 9 Liver in falciparum malaria, showing dense accumulations of small lymphocyte cells in the portal regions Note also the atrophy of the liver cells and disruption of the cell columns (From Maegraith, *Pathological Processes in Malaria and Blackwater Fever* Charles C Thomas, Publisher after Ash & Spitz, 1946.)

parasitized red cells, and haemozoin. The liver changes resemble those seen in the spleen but are less marked since a good deal of the pigment and parasite debris is removed by the spleen The hepatic lesions seem to be due to anoxia caused by interference with the return of venous blood from the liver There is probably active obstruction to efferent venous flow caused by changes in the blood vessels. This may be due to possible sphincter mechanism in the hepatic veins. There is, in effect, a stagnant anoxia Added factors may be by-products of metabolism of parasites, and possibly autoantigenesis of parenchyma cells.

Bone marrow Bone marrow changes are of the same character but not so marked as those seen in the spleen The marrow shows a well-marked erythroblastic hyperplasia with large, intensely basophilic, normoblastic cells

Brain Brain lesions occur in malaria although sometimes the symptoms and signs of central nervous system involvement clear up so rapidly and completely that one must assume temporary toxaemia or anoxia without tissue changes. Three types of lesions however may be found post mortem (1) occlusion of the capillaries and precapillaries of the cortical grey matter ring haemorrhages

around the plugged vessels and perhaps also small petechial haemorrhages in the subcortical and paraventricular white matter (2)

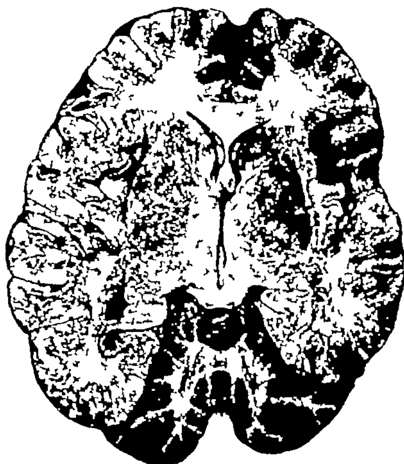


FIG. 10 Macroscopic appearance of brain in acute severe falciparum malaria. Characteristic distribution of haemorrhages. (From Maegraith, *Pathological Processes in Malaria and Blackwater Fever* Charles C Thomas, Publisher after Ash & Spitz, 1946.)

degenerative changes in brain cells with focal areas of degeneration of brain tissue (3) occasional small malaria granulomas in which a central occluded capillary will be surrounded by necrotic tissue around which, in rosette fashion, will be seen layers of glial cells and, in the outside ring many erythrocytes.

Other organs There may be intense localization of malaria parasites in any part of the gastro-intestinal tract, leading to local oedema, haemorrhages and even superficial ulceration. Occasion-

ally there is damage to myocardial fibres, and sometimes in the adrenal glands there is focal cortical necrosis.

The placenta sometimes shows very great concentration of parasites and undoubtedly transplacental infection may occur although it is surprisingly rare. Whether infection of the foetus is due to some abnormality in the placenta, which seems probable, or to active passage of sporozoites, which seems doubtful is not known. The frequency of congenital malaria has been put as high as 2 per cent in some areas, but more likely the average is much less than this.

Sometimes there is a malaria glomerulonephritis specially in quartan infections. The kidney may be swollen and the tubules may show lipoid vacuolization and hyalin droplet changes. In blackwater fever the glomeruli are usually normal, but there may be extensive changes in the renal tubules and interstitium.

Malaria pigment There has been confusion about malaria pigment. In the first place it is not melanin which is the pigment of hair skin and eyes. The true malaria pigment is *haemozoin* closely related to or identical with haematin, which is ferrihaemic acid The latter is the non-protein non-soluble iron-porphyrin fraction of the haemoglobin molecule. Haemozoin is probably free haematin It is dark red-brown in transmitted light and grey-black by reflected light. Another body pigment is *haemosiderin* which is formed by the histiocytes from any iron-containing fraction of haemoglobin It is a dark yellow pigment, probably chiefly iron in the ferric state loosely bound to protein or lipoid substances. Haemozoin possibly is slowly oxidized into haemosiderin, which in turn may be utilized by the host.

Physiologic pathology Relatively few comprehensive physiological studies have been made in malaria patients, and not much more comment than has already been made in passing can be included here. It may be noted, however that in acute malaria there is a reduction in total plasma protein, affecting mainly the albumin fraction, accompanied by an absolute increase in the globulin with a consequent rise in the globulin-albumin ratio Probably the reticulo-endothelial system has a part in this by its destruction of red blood cells and consequent liberation of much foreign material, including protein, into the plasma. This blood destruction in malaria seems also to be associated with a decrease in blood cholesterol. On the other hand there is usually an increase in blood sugar during the pyrexial stage The erythrocytic sedimenta

tion rate is increased in malaria, and, of course, in anaemia, which is so frequently a complication of malaria.

Recent observations indicate that the erythrocytic destruction stimulates a blood dilution with tissue fluids with some added protein Toxic metabolites of the plasmodium or the effects of parasite metabolism, or both tend to produce reversible alterations in the permeability of cellular membranes to Na, K and Cl. Sodium and chlorine tend to pass into the cells, so that there is a lower extra-cellular concentration of these ions, somewhat compensated for by their reabsorption by the renal tubules. The increased intra-cellular Na and Cl must produce alterations in cellular enzyme systems and other cell activity but these are not clear There is a rise in plasma potassium. There should also be mentioned a depression of the activity of the adrenal cortex in malaria. Adrenal insufficiency may occur

Observers have noted a close analogy between the signs of a malaria attack and those of anaphylactic shock. In each there is leucopenia, relative increase in mononuclear cells, lowered coagulation rate lowered arterial tension, lowered surface tension of serum with an increase of refractometric index, and similar changes in protein content of serum A few attempts have been made to desensitize malaria patients by intradermal injection of autoglobulins. This has had no effect on the parasites but seemed to hasten cessation of fever

Some evidence of hepatic dysfunction, perhaps severe is common in malaria. Kidney dysfunction may range from albuminuria to nephritis and nephrosis

Although reports are contradictory there is evidence that an individual whose spleen has been removed will have increased difficulty in coping with a malaria infection Such persons should be advised not to risk an attack.

Clinical Course

There are several types of malaria infections

Autochthonous infections are natural infections occurring through exposure to infective anophelines in endemic areas. A *congenital infection* is contracted by a foetus from its mother either antepartum or during labour In *mixed infections,* two or more species of plasmodia are present. When *falciparum* accompanies *vivax* the former will dominate the acute and the latter the chronic stage. An *induced infection* is one deliberately caused by applying an infective mosquito

or by injecting sporozoites or trophozoites or both One speaks of *sporozoite induced* or of *tropho~oite induced* malaria

The term *artificial infection* is sometimes used to describe trophozoite induced malaria and the term *natural infection* may describe a sporozoite induced malaria or an autochthonous infection The words *artificial* and *natural* in the above usage may be confusing and might better be avoided

But in all types of infection the febrile reaction follows immediately after sporulation of a sufficient number of mature schizonts to produce the necessary pyrogenic stimulation This will be modified by the immune state of the subject In autochthonous and in other sporozoite induced infections there is a delay while parasite density is building up but in trophozoite induced infections the first paroxysm may occur soon after inoculation

Incubation period The incubation period in malaria extends from the time of infection until the first manifestations of the primary clinical attack, i e arbitrarily until the first rise of fever to 100 F (37 8 C.) measured orally The *prepatent period* extends from infection to the first finding of plasmodia in the blood. The two periods are not necessarily equal nor are either of these periods exactly the same for each type of fever Average incubation periods are 12 days for falciparum malaria 13–15 days for vivax and ovale, and 28–30 days for quartan malaria Minimal prepatent periods are 5 days for *P falciparum* 8 days for *vivax* and *ovale* and 17 days for *malariae* Number of sporozoites injected, strain and species of *Plasmodium* and the resistance of the host may modify initial invasion periods.

Symptoms and signs A typical case of acute malaria will have a series of pyrexial attacks recurring sooner or later at definite intervals of 24, 48 or 72 hours. Each attack will consist of a cold a hot, and a sweating stage with a total duration in vivax malaria averaging about 11 hours. There may be a *premonitory stage* before the first rigour with lassitude, headache, anorexia aching of th bones or even vomiting

Cold stage As a typical rigour or chill begins, the patient fi feels chilly and then cold all over in spite of tropical environme extra clothes or blankets, and he may shiver and shake violen His lips are cyanotic his skin is dry and pale and has a goose-f appearance Temperature will rise several degrees and the pulse be weak but rapid. There may be headache nausea and vomi Children may have convulsive seizures. This stage lasts fro

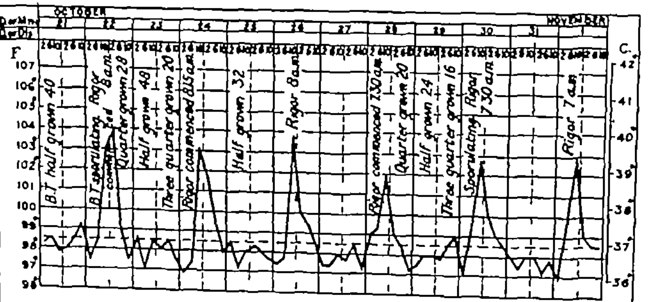


FIG. 11 Vivax malaria. No quinine treatment. Tertian periodicity (After James in Byam and Archibald, *Practice of Medicine in the Tropics*. Henry Frowde and Hodder & Stoughton, London, 1922.)

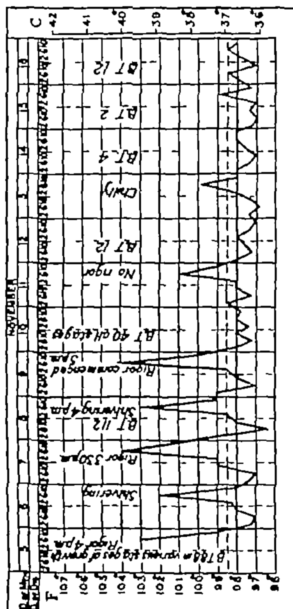


Fig. 12 Vivax malaria. No quinine treatment. Periodicity quotidian becoming tertian. (After James in Byam and Archibald, *Practice of Medicine in the Tropics* Henry Frowde and Hodder & Stoughton, London, 1922.)

minutes to an hour or longer The average duration in vivax malaria is about 40 minutes.

Hot stage Gradually the feeling of unbearable cold gives way to one of distressing warmth. The face appears flushed, eyes suffused skin hot, dry and perhaps somewhat icteric. The pulse is rapid, full, and bounding respiration rapid. There is often headache, nausea, vomiting and perhaps diarrhoea. Temperatures mount to 104° F 106° F (40° C 41 1 C) or even higher This hot stage lasts most typically for 3 to 4 hours or somewhat longer The average for vivax malaria appears to be about 4 hours.

Sweating stage Finally the patient breaks out in profuse perspiration drenching garments and bedding He is weak but greatly relieved even tranquil, and by the time he stops perspiring he may feel quite normal. Often he falls asleep although he may get up and resume his usual occupation. His temperature in the sweating stage may fall to subnormal. This stage typically lasts for 2 to 7 hours or longer The average duration in vivax malaria appears to be about 6½ hours.

There is always fever during a malarial paroxysm but the other stages may be mild in fact, so-called dumb chills present only fever In one series of cases chills were absent in about 40 per cent of the paroxysms of vivax malaria.

Attacks may come at any hour but some observers have reported that fully two-thirds of their untreated malaria patients have had their paroxysms in the morning hours between midnight and midday This is in contradistinction to septic fevers in which the temperature is more apt to rise regularly in the afternoon or evening

There is an apyrexial interval following the sweating stage, and this in classical cases will average about 12 hours in falciparum 36 hours in vivax or ovale, and 60 hours in malariae malaria. Then another paroxysm will occur Treatment may stop the acute course of the disease after one or two paroxysms, but untreated cases may continue to have typical intermittent fever for 10 days or longer

The periodic febrile paroxysms have the clinical features of a reaction to a non-specific pyrogenic agent. But in classical attacks the paroxysms are synchronous with sporulation of the parasite and it is assumed that there is a causal relationship between the rigour and the bursting of the rosettes at the culmination of erythrocytic sporogony

Types of fever Clinical malaria in practice presents three types of fever viz. intermittent, remittent, and continuous. *Intermittent*

Fig. 13 Malaria malariae. No quinine treatment. Quartan periodicity (After James in Byam and Archibald, *Practice of Medicine in the Tropics* Henry Frowde and Hodder & Stoughton, London, 1922.)

fever is characterized by regularly recurring temperature peaks related to well synchronized periodic sporulation of the parasites. *Remittent fever* displays some synchronization, as manifest by regular exacerbations in the fever curve. But there is not sufficient regimentation to prevent an almost continuous pyrogenic stimulation. The temperature remains above normal even in the remissions which alternate with the exacerbations In *continuous fever* there is no evidence of any synchronization of schizogony or of successful immune response by the host. The fever remains elevated without well-marked intermissions or remissions.

All malarial fevers tend towards intermittency most rapidly in malariae and most slowly in falciparum infections. Thus there are many clinical variations in malaria. For example, vivax malaria classically has tertian periodicity with an attack every second day But it is common to see an initial remittent fever followed by quotidian i.e. daily paroxysms specially in primary infections. Sometimes this daily attack is due to double infections, each with a brood of parasites sporulating on alternate days Sometimes the attack will come a day early and the fever is said *to anticipate* in its timing Sometimes it is postponed. If attacks are prolonged so that one has not ended when the next begins, the febrile episodes are said to be *subintrant* Sometimes the fever is remittent, not returning to normal for 2 or 3 days, and sometimes it takes an atypical continued course without chills

Quartan malaria classically has a 72 hour cycle with paroxysms every third day but there are sometimes double quartans with paroxysms on two successive days followed by a day without fever then two more peak days and so on Also one sometimes sees what appear to be triple infections of malariae, with quotidian paroxysms

Falciparum infections, with characteristic unsynchronized development of schizonts may manifest themselves in different ways. Irregularity with a prolonged paroxysm is usual There may be tertian periodicity becoming quotidian in 2 or 3 days but there are many variations of intermittent, remittent, or continuous types of fever

Splenic enlargement Splenic enlargement is typical of all malaria attacks, but usually it is not manifest in primary infections much before the seventh day As the disease continues the spleen grows in size and may become large enough to extend below the umbilicus. The enlargement is more rapid and more noticeable in infants and children. Splenomegaly develops relatively slowly in

quartan malaria it is more rapid in falciparum but is often not a conspicuous sign and it is generally prominent in vivax cases. After first infections are over the enlarged spleen may decrease in size so rapidly that it will not be palpable 2 months later But in chronic malaria or in patients who have had repeated infections the decrease in size is much less rapid.

Variations in clinical course Temperatures of 106° F (41 1 C) or even 107 F (41 7 C) are not usually serious in vivax malaria because they seldom persist for more than brief periods. Cerebral and intestinal symptoms are not common and, except during epidemics, this disease is not often fatal even when untreated.

Quartan malaria is generally no more severe than vivax and the mortality is even lower than in vivax. But nephritis appears to be a complication more often seen in malariae infections than in the others. Ovale malaria is milder than vivax but otherwise resembles it closely

Falciparum malaria, however is a different story The parasite invades more rapidly and more extensively and can multiply more quickly Moreover *P falciparum* sporulates chiefly in the capillaries of viscera where it is capable of doing more harm than in the peripheral blood stream Infections with *falciparum* must be viewed seriously because nervous system manifestations are usually due to this species of plasmodium and are responsible for a high percentage of malaria deaths. Inadequate or delayed therapy may permit serious brain involvement.

Cerebral malaria may develop gradually after headache vomiting and sighing respiration, or it may appear suddenly It may mimic acute alcoholism, tetanus or epilepsy or it may suggest acute encephalitis meningitis, or brain injury but the spinal fluid in malaria will be normal, as a rule. Symptoms may include excitement, disorientation, delirium, convulsions, somnolence, and coma. The comatose state may simulate that of encephalitis lethargica. There may be Parkinsonian effects or sometimes cerebrospinal signs and symptoms Occasionally there is a peripheral neuritis. Paralysis, even haemiplegia, occurs rarely usually clearing up completely after treatment.

Falciparum infections are also prone to cause gastrointestinal malaria, the so-called algid or cold malaria, with bloody vomitus and bloody mucous stools muscular cramps, suppression of urine and sudden collapse—suggestive of cholera except that cholera stools are not bloody So too acute falciparum malaria sometimes

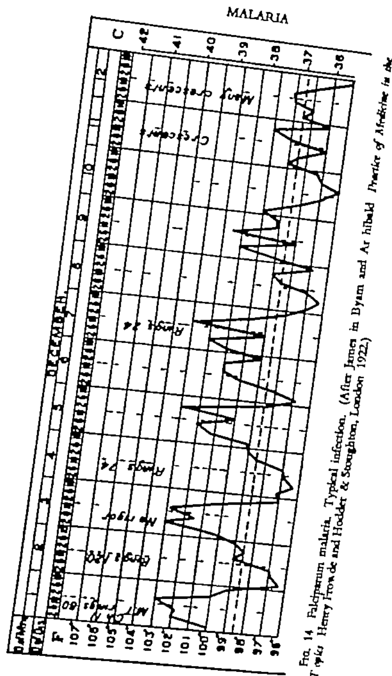


Fig. 14 Falciparum malaria. Typical infection. (After James in Byam and Ar hibald *Practice of Medicine in the Tropics* Henry Frowde and Hodder & Stoughton, London 1922.)

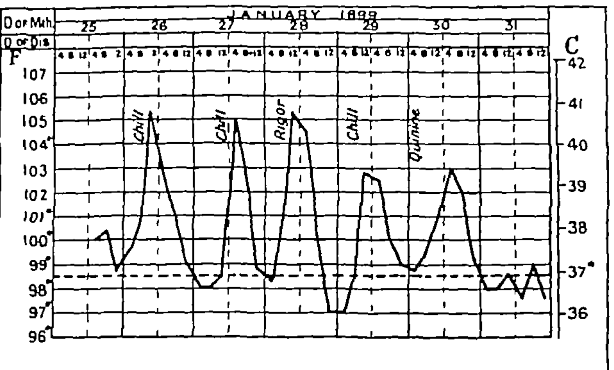


FIG. 15. Falciparum malaria. Quotidian periodicity (After James in Byam and Archibald, *Practice of Medicine in the Tropics* Henry Frowde and Hodder & Stoughton, London, 1922.)

imitates other abdominal conditions such as pancreatitis, biliary colic, dysentery typhoid fever and acute appendicitis.

Diagnosis Plasmodia seen in a blood smear have much importance in the diagnosis of malaria. Every patient in or from a malarious area should have this test, regardless of primary complaint. While no one should be deprived of treatment dictated by clinical evidence, yet repeated smears should be taken and examined in cases of reasonable doubt, and great reliance may be placed in the microscope *intelligently* used. The fever threshold in falciparum malaria varies widely from 50 parasites or less per c.mm to many thousands in those who have high tolerance. In vivax and quartan malaria the threshold is usually from 10 to 1 000 or more per c.mm. Based on the examination of 100 fields by a good technician the numerical level at which parasites are first seen in stained blood smears is about 200 per c.mm. in thin and 10 to 20 per c.mm. in thick films. In cases of doubt in the seriously ill, naturally more than 100 fields would be examined before giving a negative report. A practical measurement may be obtained by counting the parasites per 1 000 red blood cells in a thin smear making a total erythrocyte count in the usual way and then estimating parasites per c.mm (White cell counts will reveal a leucopenia, at least no leucocytosis, in uncomplicated malaria.)

Fatal untreated falciparum cases in which there were fewer than 500 parasites per c.mm. must be rare indeed. Clinically grave falciparum infections with low or negative initial parasitemia are also rare, but often a little treatment will have modified the count to bring it to a deceptively low level. Patients with over 500,000 falciparum parasites per c.mm. have survived after vigorous treatment, but apparently few have ever recovered following a density of 750 000 or more per c.mm.

Deaths from malaria have been reported after negative or weakly positive blood smears. So the patient as well as his blood smear must be under close observation specially in falciparum malaria. With or without a positive blood smear the following findings are suggestive of malaria intermittent fever enlarged tender spleen, prompt response to antimalarial therapy Spleen or sternal punctures and serological tests are not standard or very useful in malaria diagnosis

History of exposure to infection may add weight to a positive diagnosis Patients from the tropics not infrequently have chronic malaria as a complication obscured by or obscuring another infec-

tion such as dysentery Obviously a blood smear positive for plasmodia does not always mean that the patient's chief complaints are due to malaria. However the clinical course of malaria is usually characterized by intermittency and the disease has typical periods of latency and relapse. Hence one always uses caution lest an atypical symptom complex be ascribed to a disease usually typical. Nevertheless such diseases as kala azar amoebic abscess early filariasis relapsing and typhoid fevers must be kept in mind in the differential diagnosis of malaria. A physician should not allow his clinical intelligence and judgement to be blunted by laboratory findings. *Remember that to administer chloroquine or paludrine to someone free of malaria will rarely do any harm to give these drugs early in the course of malaria may do much good*

Prognosis During World War II there was a case mortality rate of less than 1 per 1 000 among U S troops treated in hospital for malaria. Vivax and quartan malaria seldom result in death except in epidemic times. But untreated or poorly treated falciparum malaria may prove fatal in up to 25 per cent of cases. When the trophozoite count on the first day of treatment exceeds 500 000 the expected death rate is over 60 per cent. Fully developed cerebral malaria is fatal in from 5 to 25 per cent of cases. When both coma and convulsions are present, the outlook is grave Multiple visceral involvement is a serious complication

Liability to relapse is discussed below

Immunity

Definitions Malaria immunity constitutes those processes in the host which prevent infection, reinfection, or superinfection or which assist in destroying the invaders or in limiting their metabolism or multiplication or which modify the physical effects of the invasion or aid specifically in repairing the damage.

Natural immunity is a species or innate character independent of previous or existing infection. For example man cannot be infected with avian plasmodia, nor can human malaria be established in birds. Here is absolute innate immunity But in other cases varying degrees of response are seen. Many negroes but few white men, for instance are highly refractory to *P vivax* again all humans are relatively insusceptible to *P knowlesi* whereas infection with this parasite often destroys a rhesus monkey Recently *P lophurae* of ducks has been established as an infection in young mice, so it is

evident that natural immunity is not as fixed a character as had been supposed

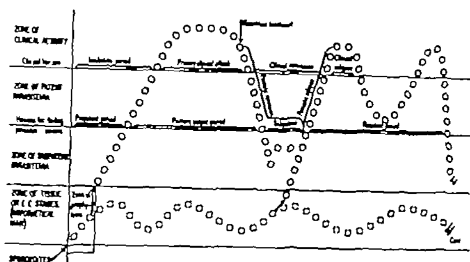


FIG 16 Diagram illustrating some relationships between malaria parasite and host. (Courtesy Drs. G. R. Coatney and W C. Cooper National Institutes of Health)

Acquired immunity arises on the basis of an existing or a past infection or as a result of some such immunizing procedure as the injection of a vaccine. It is the result of antigenic stimulation of the host by the parasite or its products

Active immunity either comes naturally due to an attack of the disease, with or without signs or symptoms or it comes artificially by inoculation of fractions of products of an infectious agent, or of the agent itself in killed modified, or variant form.

Passive immunity is attained either by maternal transfer or by inoculation of specific protective antibodies

Tolerance is immunity contingent on and conditioned by continued presence of the parasite in the host. A host with tolerance exhibits less reaction to a given quantum of infection, whereas one who has simple immunity has an increased ability to limit the quantum of infection developed. Tolerance may develop before a patient can sharply curb the parasite. *Premunition* is a term almost synonymous with tolerance, but it has a greater connotation of immunity to superinfection with the same strain of parasite. A recent attack of vivax malaria does not necessarily confer premunition and homologous superinfection is not a certain basis for testing a cure. Malaria immunity including tolerance, develops on the basis of the activity and quantum of erythrocytic plasmodia and is not directly related to the exoerythrocytic stages.

Each species of plasmodia is a complex of immunologically different strains An acquired immunity is most effective against an homologous infection but may affect heterologous strains to some extent. Anti-parasitic immunity is usually strain specific, but tolerance sometimes develops into a wider influence which may include several strains or even more than one species

When malaria is highly endemic, the unprotected indigenous people may develop and maintain a high degree of malaria tolerance through the process of often-repeated infection Completely tolerant individuals may have impalpable spleens, normal haemoglobin readings, and no symptoms although blood smears may show plasmodia This tolerance is not innate but is slowly acquired so that infants and children in such communities may suffer severely indeed a third or more may die of malaria before adulthood. Fifteen to 30 years of intense exposure may be required to develop a stable truce between host and parasite In some individuals, the tolerance becomes remarkably strong while in others it remains uncertain Although this immune process has enabled certain primitive peoples to survive in highly malarious areas in the absence of any control measures, yet it should not be overvalued so that it becomes an excuse for withholding preventive measures. Moreover while it may be true that parasitic substance, whether derived from naturally or therapeutically destroyed parasites, acts as the antigenic stimulus, yet this should not be used as an argument to delay modern treatment since a useful tolerance can be earned only by almost continuous infection with a variety of strains over a period of 15 years or more Whether or not complete tolerance to malaria occurs, with no damage to the individual young or old, is a disputed point.

Physical basis of immunity Both cellular and humoral elements are involved and are closely interrelated in malaria immunity The cellular basis of immunity is primarily phagocytosis. Macrophages dispose of large numbers of parasites parasitized red cells and malaria pigment. At first, they function at a slow pace but as the acquired immunity develops there is hyperplasia of the reticuloendothelial system and heightened activity of the phagocytic elements. Very likely also the parasite and its red cell become more susceptible through sensitization by antibodies. Opsonin-like substances, precipitins, complement fixing antibodies and agglutinins appear to be involved, but their identity and character are not clear

Thus far considerable research has not developed any method for

producing artificially an active acquired immunity in man by using killed or inactivated parasites Some passive protection may be demonstrated experimentally by injecting immune serum at the time of infection. But this has as yet no practical value.

Latency

When a malaria infection is symptomless, it is said to be *clinically latent* even though there may be a patent parasitemia and such other signs as splenomegaly *Parasitic latency* occurs when parasites are present but undetectable on standard examination of thick blood films either because for a time only fixed tissue forms survive, or because the numbers of erythrocytic forms are scanty It is known that trophozoites may be present in the blood stream in sufficient concentration so that a 5 c.c. inoculum on subpassage will infect a susceptible person yet the dilution will be such that one might examine more than a thousand thick films without finding a parasite

The term *latency* is sometimes used in referring to protracted incubation periods. For example the vivax infections of northern Europe acquired in late summer and early autumn usually have an incubation or latent period of 7 or 8 months. The first clinical episode often does not come until the following April or May

The expression *chronic malaria* includes the period of infection from the end of the primary attack to a complete or so-called *radical cure* a term which implies not only termination of infection—*parasitic cure*—but also restoration to normal health. Latent malaria presents no symptoms chronic malaria includes not only latency but also *relapses* and *sequelae* The last-named are becoming less frequent in the face of more adequate therapy and now that hook worm disease and kala azar are more readily differentiated from malaria. The principal sequela to be considered is *malarial cachexia* which is characterized by anorexia, severe anaemia emaciation and perhaps oedema, splenomegaly muscular weakness, sometimes icterus and mental apathy One still sees this condition in under-developed areas subject to seasonal or regional epidemics of malaria.

Relapse

Malaria is characterized by alternating periods of latency and renewed activity both parasitic and clinical, and the host reflects to a large extent the behaviour of the parasite Temporary interruption

in an attack due to absence of two successive paroxysms in a quotidian course, or one in a tertian course constitutes a *remission* provided activity is then resumed. The *primary attack* is arbitrarily defined as the period from the end of the clinical incubation period to the beginning of the first symptomless and fever-free period which lasts 3 weeks or longer There may be several remissions or even periods of latency during the primary attack and the latter in the absence of treatment, may persist for 2 months or more.

Clinical attacks subsequent to the primary one are called *relapses* The term *parasitic relapse* is sometimes used to indicate a reappearance of parasites or a marked increase in their numbers as noted in standard blood film examination. Some observers believe that renewed clinical activity occurring within a period of about 6 months from date of inoculation probably derives from parasitic activity which is part of the primary parasitemia Therefore they prefer to use the words *recrudescences* or *short-term renewals* for such attacks and to consider as relapses only later episodes resulting from the original infection.

Species of plasmodia differ in their tendencies to relapse. Referring to untreated cases not reinfected, one may say that falciparum malaria, if not soon fatal, tends to complete cure within 6 to 8 months with few relapses. Parasitic relapses sometimes without clinical symptoms, provide the gametocytes to initiate a new season of transmission. However there have been authenticated cases of the persistence of falciparum infections for 2 or 3 years after inoculation. Malariae infections may persist for many years even 40 or more But relapses are generally infrequent, and often the persistence of parasitemia is not realized until a blood donation results in a new case of quartan malaria.

Vivax malaria is characterized by protracted chronicity Spontaneous cure, however generally occurs within 1 or 2 years, and relapses are unusual after 3 years. Some strains of *P vivax* seem to be more prone to relapse than others, and there may be such a character as an inherent strain periodicity Moreover there is a *difference* between sporozoite and trophozoite induced vivax infections. Only recrudescences or short-term renewals of activity are usual after blood inoculated vivax malaria whereas long-term relapses are characteristic of all vivax cases acquired from sporozoites. Presumably there are fixed tissue cell forms of the parasite in sporozoite induced infections which do not occur in the other type

Ovale infections are not common and have not been so well

studied, but they appear to resemble vivax malaria as regards relapse characters.

The cause of a malaria relapse is not clearly known, and efforts to arouse one experimentally are not often successful. Short-term renewed clinical activity could be explained on the basis of a persisting erythrocytic schizogonous cycle which is held back by the immune processes of the host during periods of latency but which breaks from this restraint and produces enough parasites to pass the febrile threshold at times when the host's defences fail—for reasons still obscure. The long-term relapses of vivax malaria might arise when a continuing exoerythrocytic schizogony for some reason initiates a renewed erythrocytic cycle. One important factor in relapsing vivax malaria seems to be repeated infection involving perhaps a diversity of strains of this parasite. Evidence also indicates that the larger the inoculum of sporozoites the greater the liability to relapse and the longer the chronic period.

Therapy

Malaria therapy is complicated in several ways. (1) There are four species of plasmodia, no two equally amenable to treatment. (2) Within at least two species, *vivax* and *falciparum* there appear to be strains which have varying resistance to the same therapeutic agent. (3) The initial pre-erythrocytic, the asexual erythrocytic, and the sexual gametocyte stages differ from one another in their susceptibility to antimalarial drugs. (4) In vivax and malariae, and possibly in ovale, but perhaps not in falciparum malaria there appear to be persistent exoerythrocytic forms (as yet not well demonstrated) which may be responsible for relapses and which respond differently to chemotherapy than do other stages of the plasmodia A drug which will end an acute attack may not prevent relapses. (5) Such factors as the number of sporozoites in the infecting inoculum and the status of host resistance also influence the results of malaria treatment. The presence or absence of malaria immunity or tolerance in the patient is a relevant consideration sometimes overlooked.

Certain definitions are required in a discussion of antimalarial drugs. For instance, to many observers curative chemotherapy has implied complete suppression of acute attacks and significant lowering of the usual relapse rate but not necessarily eradication of the infection. It has been assumed that a curative antimalarial will encompass the destruction of erythrocytic stages of the parasite, but

will deal with persistent fixed tissue parasites only to a limited extent. Probably it is wiser to use the term *alleviative therapy* to indicate a régime which ameliorates or terminates an attack but does not eradicate the infection The term *curative therapy* may be reserved for treatment which completely eliminates an infection To date there is no *therapia sterilisans magna* for malaria—no antimalarial which will certainly destroy all plasmodia of all species and stages, thus resulting in complete or so-called *radical cure* Although eventually complete cure is usual in all but quartan malaria the curative process is frequently prolonged over a period of a year or two and involves immune reactions as well as drugs.

The term *protective chemotherapy* in malaria refers either to causal (sometimes called causative) prophylaxis or to suppressive treatment. *Causal prophylaxis* by definition implies the use of a drug which will bring about destruction of the one or more plasmodial stages preceding the erythrocytic forms While, strictly speaking, a true causal prophylactic should destroy the sporozoites before they take root, yet the term is also used if a drug so affects the pre-erythrocytic stages that the red cells are never invaded. *Suppressive treatment* better called *suppressive protection* or what used to be called chemoprophylaxis, consists in the administration of a drug which will act sufficiently against the erythrocytic stages to prevent the appearance of clinical symptoms, at least during the period of treatment. Such curbing of the plasmodia may be useful either to prevent clinical malaria during a period of uncontrollable exposure to infection or to prevent relapses after termination of an acute attack. *Suppressive cure* may occur during suppressive protection

Some observers have considered it useful to employ what might be called *gametocidal treatment* with a view to reducing the number of human malaria carriers in a community As will be seen in a discussion of individual drugs below *not all of them directly affect* gametocytes. However any drug which is either therapeutic or suppressive will soon bring about the disappearance of gametocytes, as well as other erythrocytic stages from the blood stream

Many drugs have been used to treat malaria but this discussion will be limited to chloroquine camoquin chlorguanide quinacrine cinchona alkaloids, and some 8-aminoquinolines. Several new synthetic antimalarials are under study perhaps the most promising being ‿ 4-diamino-5-p-chlorophenyl-6-ethyl-pyrimidine

Chloroquine

The most effective antimalarial drug at the moment seems to be *chloroquine* first made in Germany shortly before World War II. It has the following synonyms aralen, resochin resoquine, nivaquine B tanákan SN 7618 3377RP WIN-244. The drug is sold in the United States as aralen, which is chloroquine diphosphate (62 per cent base) There is also a chloroquine hydrochloride (89 per cent base) suitable for parenteral use. Nivaquine B is chloroquine sulphate.

Chloroquine appears to have no effect on sporozoites or pre-erythrocytic stages, nor does it have much, if any effect on the persistent exoerythrocytic parasites, although vivax relapses are usually delayed for 7 to 10 or more weeks after treatment. The drug does not directly affect gametocytes But chloroquine is highly effective against the erythrocytic stages of all species and strains of plasmodia of man, both as a therapeutic and as a suppressive drug While vivax and malariae infections may relapse after chloroquine treatment, falciparum malaria is usually completely cured. In adequate doses chloroquine is a good suppressive, although no drug yet available, when given in safe doses, is certain in all cases to protect against malaria. Occasional clinical cases have been reported in patients taking suppressive chloroquine.

Chloroquine localizes in the liver spleen kidneys, lungs and white blood cells. Degradation and excretion of this drug are slow plasma concentrations dropping about 60 per cent a week after the last dose. Chloroquine is not often toxic in the usual doses and it does not colour the skin. Occasionally a patient taking chloroquine may complain of blurring of vision headache, gastrointestinal discomfort, or perhaps pruritus.

Chemically chloroquine is a 4-aminoquinoline named 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline.

Therapy First dose, 1·0 gm. of the diphosphate (or sulphate) salt (0·6 gm. of base) followed in 6 hours by 0·5 gm. (0·3 gm. of base) then 0·5 gm once daily for 2 days. This amounts to 10 tablets of aralen equivalent to a total of 2 5 gm. of chloroquine salt (1 5 gm. of base) in 3 days. (The usual aralen tablet contains 0 25 gm. of the diphosphate salt. There is an army tablet of 0·5 gm. of the salt.) When circumstances indicate it, a single curative dose of 1 gm. of the salt (0·6 gm. of base) may be used best followed by suppressive treatment for 2 or 3 months

Dosage for children Total amount of the diphosphate salt over a 3-day period by age groups 1 to 6 months, 0·375 gm 7 to 9 months, 0·5 gm. 10 to 24 months 0·75 gm. 2 to 5 years, 1·0 gm. 6 to 10 years, 1·5 gm over 10 2·5 gm These totals should be divided into 6 doses 4 given the first day and 1 each on the next 2 days

Parenteral use Chloroquine hydrochloride may be given intramuscularly in a dosage of from 0·2 to 0·3 gm of base There are marketed ampoules of 3 ml. and 10 ml. of aqueous solution of the hydrochloride salt containing a total of 0·15 and 0·45 gm of base respectively Intravenous use is still experimental Doses of 0·4 gm of base in 500 ml. of sterile physiologic saline injected by intravenous drip over a period of an hour have been tried with excellent results.

Suppressive treatment Good suppressive treatment against all species and strains of plasmodium is obtained by doses of 2 tablets i.e 0·5 gm of chloroquine salt (0·3 gm. of base) taken once weekly Doses for children may be as follows age 1 to 2, ¼ tablet 2 to 5 ½ tablet 6 to 11 1 tablet 12 and over adult dose.

Camoquin

In 1946 a drug known as SN 10,751 and now sold under the trade name of *camoquin* was synthetically made in the United States. The generic name is amodiaquin, and synonyms are Cam-Aqi, CAM AQ1 and miaquin. Its chemical name is 4(3′-diethylaminomethyl-4′-hydroxyanilino)-7-chloroquinoline.

Camoquin resembles chloroquine in its pharmacology and its action on malaria. It is marketed as a dihydrochloride dihydrate in tablets, each of which contains 0·2 gm of the base. Recommended therapeutic dosage for an adult is 3 tablets, totalling 0·6 gm. of base taken as a single dose, and followed by suppressive therapy for 2 or 3 months Children from 6 to 15 years may take 2 tablets 3 to 5 years, 1 tablet 1 to 2 years ½ tablet.

Camoquin may be used as a suppressive taken at the rate of 2 tablets (total 0·4 gm. base) once a week.

Similar to camoquin is a drug of German origin, variously called *sontochin* sontoquine santochin SN 6911 and 3038RP Nivaquine C is a dihydrochloride of sontochin. Nivaquine M and R are other salts of sontochin (nivaquine B is a salt of chloroquine) There is also another quinoline *oxychloroquine* formerly SN 8137 which, although slightly less active resembles chloroquine in character and

action. But neither sontoquine nor oxychloroquine is commercially available in the United States.

Chlorguanide

Another useful and modern antimalarial is the drug called chlorguanide which has the following synonyms paludrine, proguanil B P paluril, M 4888 guanatol, drinupal, biguanal, diguanyl, chloriguane balusil, and tirian. The drug is usually sold as a monohydrochloride (87 per cent base) There are also acetate and lactate salts of chlorguanide which have been used parenterally

It is not clear that chlorguanide has any effect on sporozoites in human tissues but the drug does appear to act to some extent against the pre-erythrocytic fixed tissue stages The effect is not complete because a person inoculated during suppressive treatment may have the clinical attack after this suppressive treatment has ended. Chlorguanide has little if any effect on the persistent fixed tissue parasites. Relapses of vivax malaria will occur 7 to 10 weeks or more after treatment. This drug renders falciparum gametocytes non-infective to mosquitoes. Against the erythrocytic stages of all species, chlorguanide is quite effective, but its action is relatively slow Some strains of *P falciparum* are refractory to chlorguanide while others are sufficiently susceptible so that the disease is completely cured Unfortunately plasmodia may become resistant to chlorguanide. For example, *P vivax* on low dosages may develop an acquired resistance to chlorguanide even up to a thousandfold. Judging from recent experimental work it is important to use large doses of chlorguanide from the outset and not to be content with minimum amounts which at first will control the parasitemia.

Chlorguanide is a good suppressant against vivax malaria, but not so certain in its protective action against some strains of *P falciparum* Against neither species is a radical cure certain after suppressive treatment.

Chlorguanide localizes in the liver kidneys, white and red blood cells The drug rapidly disappears from the blood plasma, but there appears to be a degradation into active metabolites so that the duration of effectiveness of chlorguanide is not so short as might be assumed. The drug has notably low toxicity After higher than normal doses or in unusually susceptible persons, there may be nausea vomiting diarrhoea, and even mild haematuria.

Chemically chlorguanide is a biguanide named N_1-(p-chlorophenyl)-N_5-isopropyl biguanide.

Therapy 0·3 gm. of the monohydrochloride salt twice a day for 10 days. (The usual commercial tablet contains 0·1 gm of this salt.) Some observers recommend that on the first day when treating non-immunes, therapeutic amounts of a more rapidly acting drug such as quinacrine be given Other observers recommend that when malaria is treated with chlorguanide the 10-day régime should always be followed by suppressive treatment for a period of 6 weeks. Some have reported good results in vivax malaria, and in treating falciparum in semi-immune patients (e.g labour forces in the tropics) when a single dose of 0·3 gm of the drug has been followed by suppressive treatment for 6 weeks or longer

Dosage for children Children tolerate chlorguanide well. The standard formula may be used

$$\frac{\text{Adult dose} \times \text{patient's weight (in pounds)}}{150}$$

Somewhat larger doses may be used if indicated.

Parenteral use Chlorguanide acetate has been used intravenously in doses up to 400 mg and in similar dosages intramuscularly although in the latter case there is some evidence that local tissue damage may result. Parenteral administration of chlorguanide is still experimental.

Suppressive treatment Chlorguanide has been used for suppressive treatment in doses of 0·3 gm. of the salt once a week, 0·2 gm. twice a week, and 0·1 gm. daily For use after curative treatment the weekly dose may be sufficient, but for prophylaxis against acute attacks in highly endemic areas the daily dose should be taken. Even this appears to be not so certainly suppressive as chloroquine weekly or quinacrine daily

For children the following weekly suppressive doses may be given age 1 to 2 ½ tablet 2 to 5 1 tablet 6 to 11 1½ tablets 12 and over adult dose.

Quinacrine

The best-known synonyms for quinacrine are mepacrine hydrochloride B P., atabrine, and atebrin. Others are acriquine chemiochin chinacrin, crinodora, erion haffkinine italchina metoquina, metoquine, palusan arichin methoquine. The U S.P salt is a dihydrochloride dihydrate (79 per cent base) Atebrin musonat was a German trade name for diethylsulphonate of atabrine atepe a trade name for tablets containing both atabrine and plasmochin metoquina compuesta, a Latin-American trade name for atepe

Quinacrine has little or no effect on sporozoites or pre-erythrocytic stages nor does it have much, if any effect on the persistent fixed tissue parasites, although vivax relapses are delayed for 4 to 6 weeks or more after treatment. The drug does not directly affect gametocytes. But quinacrine is highly effective against the erythrocytic stages of all species and strains of plasmodia of man both as a therapeutic and as a suppressive drug While vivax and malariae infections may relapse after treatment, falciparum malaria is usually completely cured.

Quinacrine localizes chiefly in the liver spleen, lungs heart, and white blood cells. It is slowly eliminated, so that plasma concentrations drop only about 50 per cent per week after the last dose. In the usual doses quinacrine is not often toxic although it is more so than chloroquine, camoquin, or chlorguanide. Symptoms and signs of quinacrine toxicity include anorexia, nausea vomiting diarrhoea, cortical stimulation with temporary mental disturbances, and sometimes severe skin lesions. Quinacrine dyes the skin yellow but this is not a toxic or a permanent, although sometimes a disconcerting effect.

Chemically quinacrine is an acridine dye named 6-chloro-2-methoxy-9(4-diethylamino-1-methylbutylamino) acridine.

Therapy First day 0·2 gm of the salt every 6 hours, a total of 5 doses. Thereafter 0 1 gm. 3 times daily for 6 days. This provides in all 2 8 gm. of the salt in 7 days. (The usual commercial tablet of atabrine contains 0 1 gm of the salt.)

Dosage for children Quinacrine is well tolerated by children For children and frail individuals the standard formula for dosage may be used.

Parenteral use While quinacrine has been used intravenously this method is not recommended by most observers. But its use intramuscularly is a safe and effective procedure. In sterile distilled water 0·2 gm. of the drug is injected intramuscularly into each buttock with the usual precautions (total dose, 0·4 gm.) If necessary one or even two similar doses may be given at intervals of 6 to 8 hours, but as soon as oral medication is feasible it should be adopted in place of the injections. The total dose of quinacrine by both routes should be 2 8 gm. in 7 days. Initial intramuscular doses of quinacrine may be followed by oral doses of chloroquine, camoquin, or chlorguanide. For intramuscular administration of quinacrine to children or underweight patients, suitable dosage may be estimated by the usual formula.

Suppressive treatment Good suppressive treatment against all species and strains of plasmodium is obtained by daily doses of 0 1 gm of the quinacrine salt. This régime when continued for 4 or more weeks after exposure, will usually result in suppressive cure of falciparum infections. But vivax and probably malariae attacks may occur after suppressive treatment.

Quinine

The quinine alkaloid of cinchona reigned supreme in malaria therapy for 300 years but has now been dethroned by the synthetic antimalarials described above. However in relapsing vivax malaria quinine is still a useful drug when used in combination with an 8-aminoquinoline as described below Moreover in a few areas where quinine and the standardized mixture of cinchona alkaloids known as *totaquine* are prepared locally they may still be recommended in malaria therapy simply because they are cheap and do not require monetary credits in hard currency

The chemical name for quinine is 6-methoxy alpha (5-vinyl-2 quinuclidyl)-4-quinolinemethanol.

Quinine is only slightly localized in the tissues and is rapidly metabolized so that 24 hours after ingestion the drug has lost 90 per cent of its plasma concentration.

Quinine appears to have no direct effects on sporozoites or on any fixed tissue forms of the parasite It does not greatly curtail the infectiousness of gametocytes, although it does have some gametocidal effect in vivax and malariae infections. But quinine effectively interrupts the schizogonic cycle of all species and strains of plasmodia, although its action is not so rapid as that of quinacrine and chloroquine against these erythrocytic stages. However relapses are common after quinine therapy and may come as soon as 1 or 2 weeks after treatment.

Quinine often causes toxic reactions, perhaps more commonly than has been realized. But these reactions are usually mild and transitory Therapeutic doses as a rule cause cinchonism, which is manifest by such signs and symptoms as tinnitus, vertigo partial deafness, visual disturbance headache nausea, and urticaria. Rarely angioneurotic oedema has occurred Some persistent eye and ear effects, such as slight deafness and a constriction of the visual field, have been reported. It must also be noted that quinine seems to have been a precipitating factor in blackwater fever However over

centuries this drug has been extensively used by a great many individuals with no more than slight discomfort.

There are many salts of quinine, the most common being the sulphate (83 per cent base) and the dihydrochloride (82 per cent base) The latter is the most soluble and therefore is the one used parenterally

Therapy Quinine dosages used to be much larger than necessary Careful observations on volunteers have made it clear that 2 gm per day for 7 days will do all that can be done with this drug in the treatment of malaria. The usual régime is 0·65 gm. (10 grains) 3 times daily immediately after meals. It is a good plan to give a mild saline purge at the onset of treatment and to prescribe moderate alkaline therapy daily

The dosage of totaquine is the same as that of quinine.

Parenteral treatment When oral therapy is not possible or feasible, and quinacrine or chloroquine for intramuscular use is not available, quinine may be given parenterally Since quinine injected *into muscle tissue is not only a source of much discomfort but also* frequently causes local necrosis, it is preferable to administer the drug intravenously For this purpose, 0·6 gm. or 10 grains of quinine hydrochloride in some 300 to 400 ml. of sterile physiological saline is injected intravenously with not only the usual precautions but with *great* emphasis on *slow administration* Not more than a grain of quinine per minute should ever be injected into the blood stream Patients have suffered severe shock and even death apparently because of rapid intravenous injection of quinine. The dose may be repeated in 6 to 8 hours if indicated, but oral medication should be used as soon as possible. There is much to be said for giving the parenteral quinine to comatose patients by a continuous-drip method over a period of 24 hours, or longer if necessary

In an emergency when oral medication is impossible and one has no antimalarial drug suitable for parenteral use, it is possible to administer quinine by rectum. *Mix 1 or 2 gm of quinine sulphate* with starch paste thin enough to pass through a rectal catheter and run the mixture into the rectum by gravity The quinine is rapidly absorbed and one or two such doses will not generally cause serious local irritation.

Treatment of children The dosage for children or underweight individuals may be estimated on the basis of the ormula (given above) Since quinine is very bitter it may oated

Palatable liquid mixtures may be made for children by using syrups of liquorice or of yerba santa or some such vehicle

Suppressive treatment For prophylactic use no less than 0·65 gm. or 10 grains of quinine should be taken daily and this treatment which frequently permits a clinical attack is recommended only in the absence of such drugs as chloroquine camoquin chlorguanide or quinacrine.

8-Aminoquinolines

There are several 8-aminoquinolines which have been used in malaria therapy The oldest, best known and most toxic is *pamaquine* (plasmochin plasmoquine praequine, gamefar quipenyl leprochin) Chemically this drug is 6-methoxy 8-(4-diethylamino-1 methyl butylamino) quinoline. A similar drug is *pentaquine* (SN 13,276) which is 6-methoxy-8-(5-iso-propylaminoamylamino) quinoline. A third is *isopentaquine* (SN 13,274) which is 6-methoxy-8 (4-iso-propylamino-1-methylbutylamino) quinoline The latest is *primaquine* (SN 13,272) which is 8-(4-amino-1 methylbutylamino)-6-methoxy-quinoline. At the time this is written only pamaquine and pentaquine are available commercially but primaquine is to be marketed.

All of these 8-aminoquinolines are relatively toxic. But, in combination with quinine, all have a definite effect on the persistent fixed tissue stages which appear to cause vivax relapses. But they are apt to produce methaemoglobinemia, cyanosis, fever abdominal cramps, and even acute intravascular haemolysis. The combination of quinacrine or of sulphonamides and an 8-aminoquinoline is particularly liable to produce these toxic effects Quinacrine and plasmochin, for instance, should not be given together The 8-aminoquinolines are not recommended for any but specialized use under careful supervision for the specific curative treatment of relapsing vivax malaria.

Treatment of relapsing vivax malaria For this purpose pamaquine may be given 3 times a day in doses of 0·01 gm. combined with 0·65 gm (10 grains) of quinine sulphate for a period of 14 days. This amounts to 0·03 gm. of pamaquine base and 1·95 gm of quinine daily

Pentaquine or isopentaquine may likewise be given 3 times a day at 8-hour intervals in doses of 0·01 gm of base combined with 0·65 gm. (10 grains) of quinine sulphate for a period of 14 days This amounts to 0·03 gm of the quinoline and 1·95 gm of quinine

daily Some observers recommend that the pentaquine dosage be increased to 0·01 gm. every 4 hours adhering to a concurrent regimen of 3 doses of 0·65 gm quinine sulphate daily This amounts to 0·06 gm of pentaquine base and 1 95 gm of quinine daily and is close to the maximum tolerated dose of pentaquine.

Primaquine is given with quinine in the same dosages as pamaquin and isopentaquine In the case of primaquine, some observers recommend single daily doses of 0·03 gm. of base. The delayed toxicity which has been reported for primaquine seems to be avoidable in most cases by adding to the regimen theobromine in doses of 0·2 gm. once a day Adrenalin and ephedrine may also be helpful in this respect.

Treatment of Cerebral Cases

Patients with falciparum malaria should be watched carefully for such signs of impending coma as mental clouding non-purposive actions and bed-wetting If coma develops and the systolic blood pressure is over 100 mm. Hg and the pulse is good, intravenous quinine or intramuscular quinacrine or chloroquine may be given as suggested above. This medication can be repeated in 6 to 8 hours if oral administration is not then feasible. Occasionally in a large patient or very heavy infection the dose is repeated after only 4 or 5 hours. If the systolic blood pressure is below 100 mm. Hg and the pulse volume is poor then not only specific therapy but also intravenous saline, or *still* better plasma is indicated, administered rapidly at first. The quinine medication if used may be added to the last 400 ml. and *injected slowly*

Restlessness and convulsions should be controlled with intravenous *pentothal sodium or intramuscular luminal sodium.* Cardiac failure, *respiratory obstruction and cerebral oedema should be dealt with promptly if they occur*

General Treatment

In addition to specific therapy it may be useful to relieve headache and general discomfort with aspirin to administer barbiturates as sedatives, and to keep the bowels open Patients are confined to bed during an acute attack, but the treatment of vivax malaria in semi-immune individuals does not usually require hospitalization. High fever is best attacked by the specific therapy but may also be relieved by cold sponges and packs not by antipyretics. Fluid intake should be 3 or 4 litres daily given intravenously if necessary with

due attention to salt requirements Vomiting if persistent, may be counteracted by intravenous 5 per cent glucose in physiologic saline 200-400 ml supplemented by 1 mg of thiamine hydrochloride for each 25 gm of glucose. Occasionally blood transfusions are useful if anaemia is severe.

Delirious patients must be guarded to prevent self injury More than one person with severe malaria has jumped to death from a sickroom window or steamer deck

In convalescence, give a high vitamin high protein diet supplemented by ferrous sulphate 0·6 gm (10 grains) after each meal for a month or more as indicated.

Blackwater Fever

Probably blackwater fever is simply malaria with haemoglobinuria although the precise relationship has not been proved. The condition is usually limited to those who have had repeated intense infections, most often treated poorly with quinine. More recently blackwater fever has been reported after quinacrine therapy in West Africa. Characteristically it appears in the non-indigenous population of a highly endemic area where *P falciparum* predominates affecting most commonly individuals who have been in the area for 6 months or more. Possibly some immune reaction with an explosive output of haemolysin is involved in the production of the haemolysis, leading to excessive haemoglobinemia, haemoglobinuria, kidney damage, and perhaps renal failure.

The course of blackwater fever may be from mild to fulminating, with prognosis varying in different series of cases from 10 to 50 per cent probable mortality

As to treatment, a full course of chloroquine camoquin or paludrine should be given if parasitemia is present. Quinine quinacrine and the 8-aminoquinolines should be avoided Cardiovascular failure should be combated and careful attention paid to fluid intake and output, maintaining a proper balance if possible The urine should be kept alkaline, but care is required to avoid an alkalosis. Blood transfusion should not be given in toxic anuric cases but whole blood is sometimes helpful in toxic polyuric patients and in the asthenia following blackwater fever Persons who have had blackwater fever should be advised to stay out of areas where malaria is highly endemic, to avoid quinine quinacrine and 8 aminoquinolines and to take careful precautions to prevent being bitten by *Anopheles* mosquitoes whenever they must be in endemic areas.

So far as is known malaria plasmodia of man may develop in almost any *Anopheles* but not in other mosquitoes and not in any other arthropod Anopheline mosquitoes are, therefore, the *biological vectors* the *definitive hosts* of *Plasmodium vivax malariae falciparum* and *ovale* (The vectors of certain bat and lizard plasmodia are not known but in the case of the latter mites have been suspected.)

Anopheline mosquitoes have the following position among the arthropods

Phylum—*Arthropoda* chitinous exoskeleton paired, jointed appendages.

Class—*Hexapoda* the insects (over 750 000 species) distinct head, thorax, and abdomen thorax with 3 segments, each bearing a pair of legs many species also possess wings.

Order—*Diptera* the flies (some 85 000 species) usually a single pair of wings on second thoracic segment knobbed halteres on third segment sucking mouth parts egg larva, pupa, adult stages.

Suborder—*Nematocera* slender bodies many jointed antennae adults emerge from pupal skin by dorsal slit.

Family—*Culicidae* true mosquitoes and their near relatives (*Chaoborinae*) narrow scaled wings aquatic larvae and pupae. (Over 1 500 species in the family)

Subfamily—*Culicinae* true mosquitoes only functional proboscis which, in females of all but a few species, is adapted to sucking blood wing veins heavily scaled 3 tribes, *Anophelini Megarhinini* and *Culicini*

Tribe—*Anophelini* proboscis not rigid scutellum usually rounded palpi long in both sexes 3 genera, *Anopheles* Meigen 1818 *Chagasia* Cruz, 1906 and *Bironella* Theobald, 1905 (Some 400 species in the tribe.)

Genus—*Anopheles* all have rounded scutellum most have spotted wings. Several subgenera have been described.

Characters of a Malaria Vector

While it is true that a percentage of almost any species of the *Anopheles* genus can be infected with plasmodia in laboratory tests

by experienced workers, yet many species are not natural or are poor ones. Zoologic relationship even close within

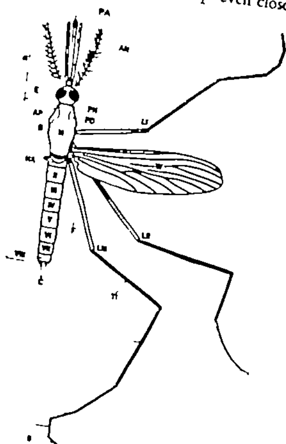


FIG 17 Adult female *Anopheles*, dorsal diagram *A*, abdomen with segments I to VIII *T* thorax *H* head *AN* antenna *PA*, palpus *L*, labium or proboscis *E*, compound eye *PN* pronotum *AP* anterior promontory *FO* fore... *M*, mesonotum *S* scutellum *HA*, haltere *P* postnotum *C*, cerci *LI* foreleg *W* wing *LII* middle leg *LIII* hind leg *F* femur *TI* tibia *TA*, tarsi 1-5 tarsal segments (From Russell, Rozeboom, Stone, *Keys to the Anopheline Mosquitoes of the World* American Entomological Society Philadelphia, 1943 after Gray 1935)

subgenus, appears to have little significance as regards the practical malaria transmitting importance of a given species.

To be an effective vector a species must be present in reasonable numbers in or near human habitation An anopheline that is too shy to enter man s dwellings or that prefers high altitudes, or dense jungle or other relatively uninhabited places, is not apt to be dangerous. Secondly a species characterized by a marked preference

for animal rather than human blood is less apt to propagate human malaria. Of course, under certain circumstances it may be so abundant, or its preferred host so scarce, that relatively large numbers are forced to take man's blood. Thus it becomes an unusual vector. Again, a mosquito that cannot remain alive long enough for the completion of the sexual development of the plasmodium within its body cannot become a vector. It is the infective, not the merely infected, mosquito which menaces man. Thus, length of mosquito life must be a factor in malaria transmission, and no doubt this varies in different seasons and places with temperature and relative humidity. A species that can resist desiccation better than another will be more apt to transmit malaria in relatively dry areas or seasons.

Finally, there is the varying susceptibility of individual anophelines to infection, and this depends on hereditary factors and probably also on biochemical characters of ingested blood and on the metabolic and nutritional requirements of plasmodia.

Morphology

Some attention must be given to mosquito morphology to provide a basis for differentiating species, for dissecting to find sporozoites or oocysts, and for understanding the natural history of *Anopheles*.

Adult anopheline. *Head*, *thorax*, and *abdomen* of an adult mosquito are shown diagrammatically in Figure 17. Each abdominal segment consists of dorsal plate or *tergite*, a ventral plate, or *sternite*—both of chitin and connected by a membranous *pleuron*. The diagram shows *antennae*, *palpi*, *proboscis*, *compound eyes*, *wing* (only one drawn), *halteres* or "balancers," *mesonotum*, *scutellum*, and *legs* (only three drawn). Each leg has *femur*, *tibia*, and 5-segmented *tarsus*. The palpi are about as long as the proboscis in all male mosquitoes and in most female *Anopheles*. In female culicines, palpi are much shorter than proboscis.

The main visible part of the proboscis is called the *labium*, and it terminates in a pair of *labella*. The labium is a sheath for the mouth parts. The latter include *labrum*, a pair of *mandibles*, the *hypopharynx*, and a pair of *maxillae*. These 6 chitinous structures are called *stylets* or, collectively, the *fascicle*, and in females they are suited to the penetrating of skin and the sucking of blood. The mandibles and

maxillae are serrated and sharpened for cutting and piercing The hypopharynx forms a common salivary tube into which empty the

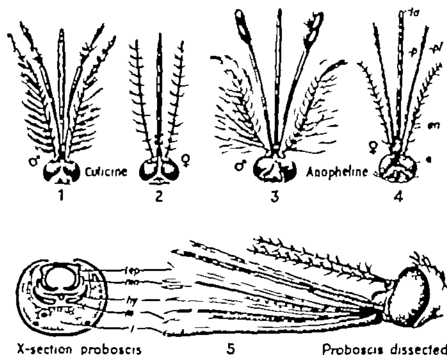


Fig 18 Mosquito head and appendages *an*, antenna compound eye *hy* hypopharynx *l* labrum *la*, labellum *l p* labrum-epipharynx *m* mandible *m* maxilla *p* proboscis *pl* palpus (After Russell, West Manwell, *Practical Malariology* W B Saunders Co Philadelphia, 1946)

ducts from the two salivary glands. Mosquito mouth parts are illustrated in Figures 17–19

The wings have characteristic *venation* shown in Figure 20 Arrangement and colour of the scales on wings palpi, legs and on the body may be useful in classification of species.

The terminal segments of the abdomen are modified for purposes of mating and in the female also for ovipositing The male characters are useful in taxonomy (See Figure 21)

The *alimentary tract* of a female mosquito is shown in Figure — Note the *pharyngeal pump* that creates suction which, added to a possible capillary pressure, draws the fluid meal through the labrum A *salivary pump* forces saliva down the hypopharynx. Also drawn in Figure — are the *dorsal* or *oesophageal diverticula* sometimes mistaken for salivary glands There is a third or *ventral diverticulum*

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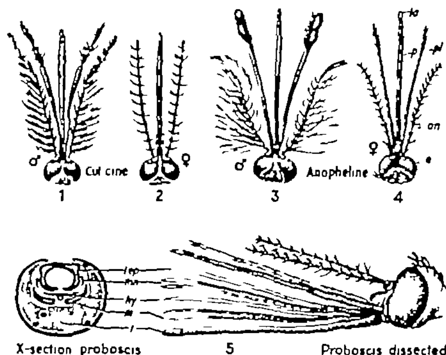


FIG. 18. Mosquito head and appendages. *an* antenna, *ce* compound eye, *hy* hypopharynx, *l* labrum, *lb* labellum, *l-ep* labrum-epipharynx, *mn* mandible, *m* maxilla, *p* proboscis, *pl* palpus. (After Russell, West, Manwell, *Practical Malariology*. W. B. Saunders Co. Philadelphia, 1946.)

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sometimes confused with the stomach. The function of these diverticula, which appear as glistening hyaline sacs, sometimes referred to as the insect's *crop* is not known, although they seem to take up any air swallowed with the food. The paired *salivary glands* are in the anterior ventral part of the thorax. Each has 3 and sometimes 4 lobes. *Fore-* *mid-* and *hind-gut* as well as *rectum* may be differentiated. Blood digestion and the early sexual stages of development of plasmodia occur in the mid gut, which is also known as the *stomach* Oöcysts and sporozoites develop on the wall of this organ.

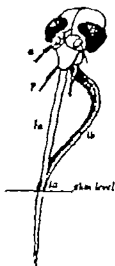


FIG. 19 Head of female feeding and p. tenn c a d palps (broken off in diagram) fa fascicle or bundle of stylets la, labella lb labium (After Weber Lehrbuch der Entomologie Jena, 1933)

There are 2 gonads, *ovaries* or *testes* in the posterior part of the body cavity In the female there is a *spermatheca* which is a globular structure lying within the eighth abdominal segment. In this sac the spermatozoa are stored after the insect has mated. During the life of the female the *sperm cells from a single mating* may remain alive in sufficient numbers to fertilize all the batches of eggs which mature.

Anopheline eggs The eggs of *Anopheles* are laid singly on water They are shaped like tiny boats or canoes pointed but with the head end a little wider The mean length, after some growth in the first 24 hours, is about 0 5 mm—a speck just visible to sharp eyes (Figure 23) The gunwale edge of the egg is a more or less striated frill which may or may not be continuous. There are also cell-like floats opposite the mid portion on each side, and made up of transparent exochorion. The endochorion is also transparent at first, but soon darkens, and the grey exochorion forms a pattern against the brownish-black endochorion which is the inner wall, or egg shell. This pattern may be useful in species differentiation So too egg contours may be unique in some species

Anopheline larva Mosquito larvae ('wigglers or wrigglers') have distinct head, thorax, and abdomen. Each of these sections carries hairs many of which are useful in taxonomy Figure 24 shows the following important hairs *outer clypeal* *inner clypeal* *posterior clypeal* *antennal* *subantennal* *sub-basal* *frontal* *outer occipital*

ans-sutural and *inner occipital* or *sutural* In Figure 25 are shown am thoracic hairs numbered according to standard and well-

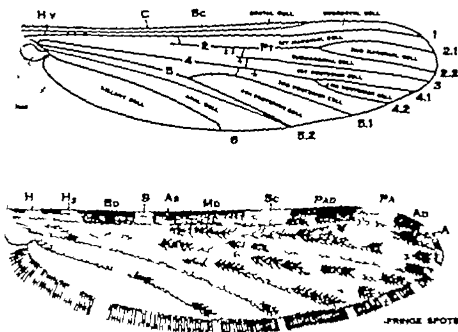


FIG 24 Upper diagram, wing of *Anopheles* illustrating venation *Hv* humeral crossvein *C*, costa *Sc* subcosta *Pt* petiole of vein 2 1 2 1 2 2, 3 4 1 4 2, 5 1 5 2, and 6, these are the longitudinal veins and their branches The wing cells are named in the diagram

Lower drawing, wing of *A gambiae* illustrating diagnostic wing spots *Pale spots* *H H* H_3 humeral spots *S* sectoral spot *Sc* subcostal spot *Pa*, preapical spot *A* apical spot *As*, accessory sector spot *Dark spots* *Bd*, basal dark spot *Md*, median dark spot *Pad*, preapical dark spot *Ad*, apical dark spot

(After Ross and Roberts, *Mosquito Atlas* American Entomological Society Philadelphia, 1943)

known practice among taxonomists The same is true of Figure 26 which shows abdominal hairs.

Other morphological points about the larva are the simple *alimentary canal* and the specialized *caudal segments* On the latter culicine larvae carry a more or less conspicuous *breathing syphon* not present in anopheline larvae which, however have the usual two spiracular openings leading to the *tracheae* (Figure 27)

It may also be noted that larvae have conspicuous *mouth brushes* which by oscillatory movements sweep food particles into the mouth. The body normally lies parallel to and just beneath the water surface In order to feed at the surface the larva rotates its head 180 Culicine larvae hang head downwards and generally

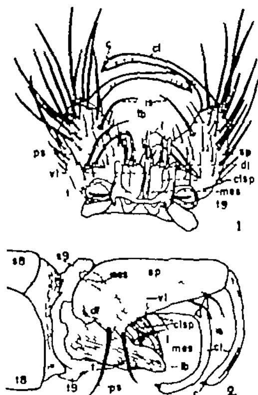


Fig 21 Male genitalia, *A. quadrimaculatus*. 1 Structures viewed from lower side. 2, Lateral view (The morphologically dorsal parts have become rotated into a lower or ventral position in the adult.) *c* claw or spine of clasper *cl*, clasper *clsp*, claspettes *dl* dorsal claspette lobe *is* internal spines *l* leaflets of mesosome *lb* anal lobe *mes*, mesosome (phallosome) *ps*, basal (parabasal) spines *sp* side piece (coxite) *vl* ventral claspette lobe *s8* and *s9*, 8th and 9th sternites *t*, lobe of 9th tergite *t8* and *t9*, eighth and ninth tergites. (After Russell, West, Manwell, *Practical Malariology* W. B. Saunders Co Philadelphia, 1946 after Matheson.)

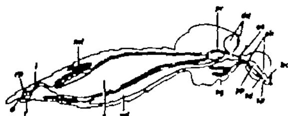


Fig 22. Alimentary tract of female mosquito *a*, anus *bc*, buccal cavity *dd*, dorsal diverticula *i* intestine or hind gut *mt* Malpighian tubules *oe* oesophagus *ph* pharynx *pp* pharyngeal pump *pr* proventriculus (foregut) *r* rectum *rp* rectal papillae *s*, stomach (midgut) *sd*, salivary duct *sg* salivary gland *sp* salivary pump *vd*, ventral diverticulum. (After Russell, West, Manwell, *Practical Malariology* W B Saunders Co., 1946.)

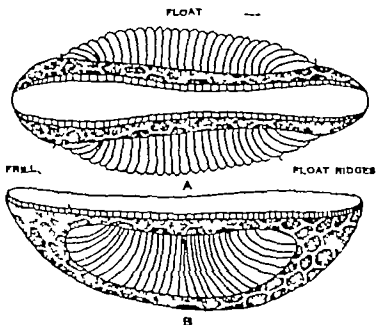


Fig. 23. Egg of *A. gambiae*. *A*, dorsal view. *B*, lateral view. (After Russell, Rozeboom, and Stone. American Entomological Society, Philadelphia, 1943; after Gibbins, 1933.)

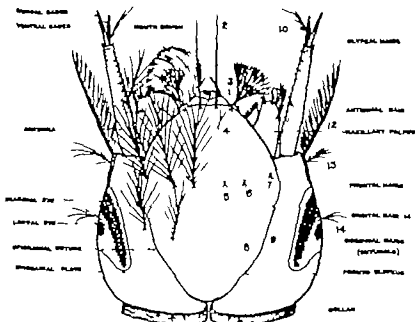


Fig. 24. Head of *Anopheles* larva, dorsal view showing following hairs: 1, preclypeal; 2, inner clypeal; 3, outer clypeal; 4, posterior clypeal; 5, inner frontal; 6, middle frontal; 7, outer frontal; 8, inner occipital; 9, outer occipital; 10, terminal antennal hair; 11, antennal hair; 12, subantennal hair; 13, hair from side of epicranial plate; 14, orbital hair. (After Ross in Russell, Rozeboom, and Stone: *Keys to the Anopheline Mosquitoes of the World*, American Entomological Society, Philadelphia, 1943.)

feed on sub-surface particles. Another point of interest is that mosquito larvae undergo 3 ecdyses when they shed their chitinous

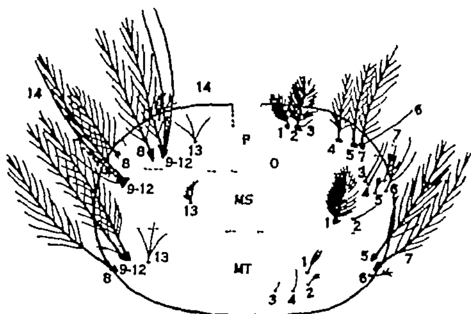


FIG. 25 Thorax of *Anopheles* larva, dorsal view on right, ventral view on left. *P* prothorax *MS* mesothorax *MT* metathorax. Prothorax, dorsal *1* prothoracic hair 1 *2*, prothoracic hair 2 *3*, prothoracic hair 3 Prothorax, ventral *9* to *12*, hairs of the prothoracic pleural group *13* innermost hair on ventral surface. Mesothorax, ventral *9* to *12* hairs of mesothoracic pleural group Metathorax, dorsal *1* metathoracic palmate hair Metathorax, ventral *9* to *12*, hairs of metathoracic pleural group Other unnamed thoracic hairs are referred to by number (After Russell, Rozeboom, and Stone, *Keys to the Anopheline Mosquitoes of the World*, American Entomological Society Philadelphia, 1943 after Gater 1934)

skin These molts separate the life of a larva into 4 stages or instars, each larger than that preceding At the end of the fourth stage, the larva pupates.

The chromosomes of the cells of the proximal lobe of the salivary gland of *Anopheles* larvae appear to have patterns which are distinctive by species of mosquito Comparative karyology in this respect may eventually prove useful in making possible a differential diagnosis of closely-related and otherwise morphologically-similar species in certain complexes like those of *maculipennis pseudopunctipennis punctulatus* and *tarsimaculatus*

Anopheline pupa The pupa or tumbler requires little detailed description here. Attention is called to the conspicuous funnel-shaped *respiratory trumpets* on the head (not tail, as in larva) Certain pupal hairs are sometimes used for taxonomic purposes.

Classification

The following key will help to separate *Anopheles* adults from other mosquitoes and flies

1 Wing venation as in Figure 20 wing veins have scales attached mouth parts long tubular and adapted for piercing 2
Without this combination of characters 3

2. Female palpus almost as long as proboscis (except in *Bironella*) abdomen never entirely covered with scales often nearly bare (lateral or ventral scale tufts may be present) scutellum crescent-shaped (except in *Chagasia*) with evenly-distributed marginal hairs Tribe *Anophelini*

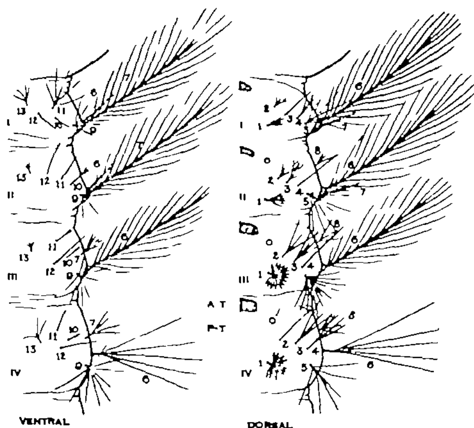


Fig. 26. Abdominal segments I–IV of *A. quadrimaculatus* larva. *I* to *IV*, abdominal segments; *A-T*, anterior tergal plate of segment IV; *P-T*, posterior tergal plate of segment IV; O, the small hair No. 0 on dorsal surface; 1, abdominal palmate hair; 2, antepalmate hair; 6, lateral hair. Unnamed hairs are referred to by numbers. (After Ross, in Russell, Rozeboom, and Stone, *Keys to the Anopheline Mosquitoes of the World*. American Entomological Society, Philadelphia, 1943.)

Female palpus much shorter than proboscis abdomen with both tergites and sternites densely covered with flat scales scutellum usually trilobed 4

3 Not in the *Culicinae* sub-family

4. Mosquitoes not in the *Anophelini* tribe.

The following key separates anopheline larvae from other aquatic insect larvae

1 Head in a distinct capsule 2
Head not clearly distinct from other body segments 5

2 Three thoracic segments fused to form a more or less dilated mass considerably wider than any abdominal segment 3
Three thoracic segments distinctly annulated or segmented, each having general appearance of an abdominal segment 5

3 Antennae not fitted for grasping and not equipped with long stout apical spines 4
Antennae fitted for grasping and equipped with long stout apical spines 6

4. No elongated air-tube syphon Tribe *Anophelini*
Elongated air-tube syphon which is at least as long as it is broad and is located on eighth abdominal segment
Tribe *Megarhinini* or *Culicini*

5 Not in *Culicidae* family

6 Not in *Culicini* sub-family

Physiology

Anopheline larvae and pupae require atmospheric oxygen and are asphyxiated if they cannot obtain it. Some *Culex* larvae can take enough dissolved oxygen through their skin to survive in well-aerated water even after the tracheae are plugged with a non-toxic oil.

Mosquito pupae, although very active, do not feed Larvae take a mixed diet of gross and microscopic food particles varying in size from about 20 to 100 microns in diameter Bacteria, infusoria algae, and other material without much apparent selection are swept in by the mouth brushes. Larvae can utilize colloids or nutrients in solution provided the necessary vitamins and mineral salts are present.

Adult female mosquitoes normally feed on blood The amount of blood taken depends somewhat on size of insect and its development As examples, *albimanus* a small mosquito is reported to

average from 0·8 to 1 3 mg per meal while *quadrimaculatus* may take up to 3 – mg and probably averages about 2 5 mg In one set of measurements with the last-named species, the virgins meals averaged – 7 mg., while those of the gravid individuals averaged –·0 mg The body weights before feeding averaged 1 73 mg and –·65 mg respectively Blood meals are completely digested in from – days to – weeks depending on temperature. The insect absorbs the globin but excretes the haeme of the haemoglobin

The nature of mosquito saliva is not well known but it may contain substances which stimulate capillary dilation or which slow coagulation There is an immediate allergic response in persons who have been sensitized Unsensitized and de-sensitized individuals do not have the usual mosquito-bite reaction The substances causing this response seem to be highly specific for different mosquitoes. There may also be a delayed papular reaction due it seems, to a slow acting toxin—not to sensitization.

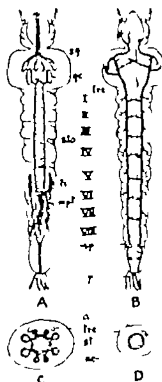


Fig. 77 Mosquito larva, diagram of certain internal organs A, alimentary canal and testes B dorsal heart and tracheae C cross section through gastric ca D cross section through abd m l segment IV an rt ap l papillae gc gastric caeca mpt, Malpighian t bes er d g salivary gland sp spiracle sto, stomach tr tracheae ts, testes. (Af B yd Af l l l gy W B Saunders Co 1949 modified from Brumpt, 1936, and Marshall, 1938)

Insects have a complex of enzyme systems about which little is known For instance, tyrosinase seems to aid in the formation of cuticle and the deposition of melanic particles. But tyrosinase represents only one aspect of the subject. Why for instance, will all stages of *P vivax* normally be completely digested by *Aedes aegypti* whereas gametocytes of *P gallinaceum* taken into the gut of the same insect will not be digested but will be stimulated to proceed with sexual development?

Adult male mosquitoes normally feed on plant juices. They are unable to penetrate skin but can be made to suck liquid blood in a laboratory Both males and females will develop on a diet of sugar and water But on such a diet most females will not mature their

Female palpus much shorter than proboscis abdomen with both tergites and sternites densely covered with flat scales scutellum usually trilobed 4

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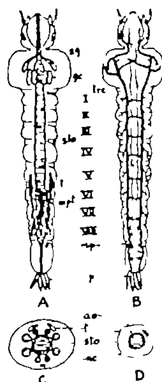


Fig 27 Mosquito larva, diagram of certain internal organs A, lmentary anal and testes B, dorsal heart and tracheae C, cross section through gastric caeca D cross section through bd m l segment IV t ap l papillae gc gastric caeca mpt Malpighian t bes rv d f salivary gland sp spiracle st stomach tr tracheae te, testes (Aft B yd M l legy W B Saunders Co 194) modified from Brumpt, 1936 and Marshall, 1938)

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Adult male mosquitoes normally feed on plant juices They are unable to penetrate skin but can be made to suck liquid blood in a laboratory Both males and females will develop on a diet of sugar and water But on such a diet most females will not mature their

eggs. With some few exceptions blood meals are required. Mosquito species may react differently to various kinds of blood meals by laying more eggs while on a diet of rabbit blood, for instance, than when feeding on human blood. No one has satisfactorily explained this or most other aspects of the metabolism of mosquitoes.

As cooler weather approaches in temperate climates, females of some anopheline species prepare for hibernation by developing *fat bodies* instead of eggs. This has been called *gonotrophic dissociation*, and the period of reproductive inactivity the *diapause*

Mosquitoes are well equipped with sensory organs so that they appear to perceive light and dark, some measure of form degrees of heat and cold, certain ranges of humidity and a number of odours.

Natural History

Under natural summer or hot climate conditions, most anopheline eggs hatch within [illegible] to 3 days of oviposition However the same



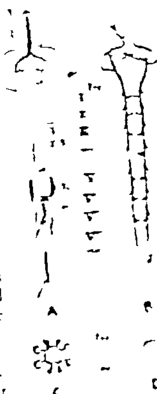
Fig. 28 Breeding place of *A superpictus* in Albania.

range from 0·8 to 1·3 mg per meal ... up to 3 mg and probably averages ... measurements with th... la... d... es, the virgins meals averaged ... while those of the gravid individuals averaged ... mg. The body weight before ... ding averaged 1·73 mg and ... mg respectively. Blood meals are compl... gested in from 2 days to ... weeks depending on temperature. The insect absorbs the globin but excretes the haem of the haemoglobin.

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Adult male mosquitoes normally ... unable to penetrate skin, but can ... laboratory. Both males and females ... and water. But on such a ...



perish if they are removed from water or are not permitted to have their breathing spiracles in contact with air

Environment Various anopheline species differ greatly in their relations to the environmental characters of their larval habitat. Chemistry and plankton of water its temperature and movement, the flora and fauna and the degree of sunlight—all have importance. No doubt *preferential breeding habits* stem much more from the ovipositing instincts of the adult females than from the behaviour patterns of the larvae, but generally they are described in relation to the latter For instance such larvae as those of *crucians* in the United States are usually collected in acid water while other larvae, like those of *punctipennis* in the same areas, are mostly taken from alkaline waters. Again species like *culicifacies* in India occur normally in fresh water others, like *sundaicus* also in India, in brackish water with a salinity of some 5 to 8 parts per 1 000 and still others such as *multicolor* in Egypt, are frequently found in water containing as much as 35 parts per 1 000—saltier than the sea (The concentration of salts in sea water averages about 33 3 parts per 1 000) But some normally fresh water species like *farauti* in the New Hebrides will be found in strongly brackish water at times. So too while as a general rule *Anopheles* larvae are found in clean water sometimes those of *subpictus* for example, are abundant in such polluted places as pig or buffalo wallows, usually harbouring only *Culex* species. Some like *darlingi* in Brazil, seem to require water with an oxygen content of 6·5 to 9·5 p p m. whereas others, like *strodei* in the same region, will do fairly well at tensions of 1 6 p p.m.—a low figure which, however does not disturb certain *Culex* species

Temperature of water of larval habitat is an important factor although, of course, one must remember that the principal environmental components interact so that in nature it is seldom possible to describe a single factor as completely decisive in its influence. However different *Anopheles* apparently have different zones of preferred or of most favourable water temperatures, and these preferences may affect distribution. Sometimes slight differences are noted. For example, it appears that the most favourable water temperature for *atroparvus* in Italy is an alternation of 25 C. (77 F) and 30° C. (86° F) while for *labranchiae* in the same country it is 25 C. and 35° C. (95 F) In other cases the difference is marked as between the Indian species *gigas gigas* always in cool water at high altitudes,

and *varuna* in warm lowland pool water. Limiting temperatures vary considerably. For instance larvae of *claviger* in Europe and



FIG 30 Breeding place of *A. sundaicus* in Java

punctipennis in America may be taken from water under ice but they are killed if frozen, unlike certain *Culex* species such as the North American *Wyeomyia smithii* whose larvae hibernate in ice in pitcher plants. The thermal death point of anopheline larvae may vary slightly by species. It has been recorded at 42° C (107·6° F) for *gambiae* and probably few if any species could survive any higher temperature. All anopheline larvae tend to develop more rapidly at higher than at lower temperatures but in some cases as with *stephensi* in India, when the water temperature is maintained at 37° C. (98·6° F) the larvae having grown rapidly die before pupating.

Some larvae, like those of *philippinensis* in India, are not usually found in running water while others like *minimus* are usually found in streams, although in places where the effects of the current, whatever these may be, are indirect. No anopheline larvae can withstand currents much in excess of about 0·9 ft. per second, and the stream breeders seem no more capable in this respect than the pool breeders.

The relation of emergent, floating and submerged vegetation to anopheline larval incidence and density has had much study. A few types of plants seem to be actually inimical to larval breeding or else to be indicators of unfavourable conditions. For instance *quadrimaculatus* in America is seldom found in association with the

plant called water-shield (*Brasenia*) In India a dense growth of duckweed (*Wolffia*) may completely inhibit mosquito larvae, while a growth of *Pithophora* often appears to favour the development of *culicifacies* There are a few special relationships as, for example in the case of *bellator cruzi* and *homunculus* in Brazil and Trinidad, found in water in the leaf axils of certain species of epiphytic bromeliads growing high up on trees or near the ground attached to rocks. Some anophelines like *barberi* in America, *plumbeus* in Sardinia, and *culiciformis* in India are found only in the water of tree holes.



FIG. 31 Breeding place of *A. minimus flavirostris* in Palawan, the Philippines.

The fauna of a mosquito breeding place may be of interest if it includes such natural enemies of larvae as the top feeding minnows (*Gambusia*) or as some predaceous insect larvae like those of certain *Megarhinus* (*Toxorhynchites*) *Lutzia Armigeres* and *Psorophora* mosquitoes, and of dragon-fly and dytiscid-beetle larvae.

As regards sunlight, some *Anopheles* larvae such as *pattoni* in China and *maculatus* in Malaya, seem to be strongly heliophilic ('sun-loving') Others, like *umbrosus* in Malaya and *leucosphyrus* in Borneo are strongly heliophobic ('sun-fearing') The rest, like *culicifacies* and *stephensi* in India, appear about as frequently in moderate shade as in bright sunlight. Indeed, *stephensi* larvae may be found in a sunny roof-top collection of very warm water and in a dark cistern of cold water in the same building on the same day Heliophobic larvae transplanted to sunny places and heliophilic larvae to shaded water seem to develop normally

Growth The rate of growth of larvae probably depends on temperature plus inherent or genetic characters of species plus the conditions of the larval habitat, specially in regard to food supply In the tropics and in hot summer weather in sub-tropic and temperate regions, most anophelines will go through the larval stage in

from 8 to 12 days. Occasionally as with *culicifacies* in Madras in some breeding places the larval stage has been as short as 6 days



FIG 3. Collecting *Anopheles* eggs W ter skimmed from surface then poured slowly through cloth mitten worn on left hand Eggs appear as typical dark specks and are transferred to cotton in small vials

Under cooler conditions the larval period may be much longer indeed, it may last all winter as often with *claviger* in Europe.

Classification of larval habitats Larval habitats may be conveniently classified as follows

I *Permanent or semi-permanent standing fresh water*

1 Large marshes, or marshy zones in lakes.
2 Small ponds, pools, borrow pits, stagnant canals and ditches.
3 Spring-fed pools such as lime sinks hot springs and seepages.
4 Standing agricultural water as in rice fields, date plantations or in waste overflow collections.
5 Wells.
6 Swamps. These differ from marshes in being inundated forest rather than inundated open country
7 Forest pools. Differ from other pools in being well-shaded

II *Transient fresh water ground pools*

1 Open pools as in fields or stagnant stream beds.
2. Pools in cart tracks, hoof prints and the like.

III *Permanent or semi-permanent running fresh water*
1 Open streams in association with vegetation.
2 Open gravel stream beds.
3 Canals and ditches.
4. Forest streams

IV *Container habitats*
1 Rock holes
2. Tree holes.
3 Special plant association as *bellator* in epiphytic bromeliads.
4. Containers such as shells gutters, tins.
5 Crab holes and mud cracks (sometimes brackish)

V *Brackish water*
1 Brackish marshes ponds, and swamps, not tidal.
2 Tidal swamps.
3 Small accumulations of brackish water such as oases pools.

Larval behaviour Larvae move by jerks of their bodies and by propulsion of their mouth brushes, and they travel tail foremost. Some anopheline larvae have been reported to crawl a few centimetres on surfaces which do not absorb moisture, as, for example,



Fig 33 Collecting *Anopheles* larvae in borrow pit, using long-handled dippers.

on wet mud from one hoof print to another after the first had become nearly dry

At rest, anopheline larvae almost invariably are found suspended from the surface film and parallel to it touching surface debris or vegetation at the edge of the breeding place. Sometimes the particles to which they cling are not much larger than themselves This tendency to contact something has been referred to as *thigmotropism* or *thigmotaxis* but is probably less a tropism than a mechanical effect which any larva-sized particle might exhibit. Larvae dive when stimulated by sudden increase or diminution in light, or by a jarring effect. They may exhibit *letisimulation* at the bottom or in simple words, they may seem to feign death holding a quiet, rigid pose. For instance it is sometimes difficult to tell which *darlingi* larvae in a collecting dipper are dead and which are playing possum There are species differences in such behaviour patterns as well as in orientation to light, current, temperature, and chemical stimuli.

The Pupa

Mosquito tumblers are the most active of all insect pupae. They respond quickly to such stimuli as light changes raindrops, and vibrations, including those of sound. When undisturbed, they tend to remain quietly in one spot as they have no reason to move. No food is taken during the pupal stage which normally lasts 1½ to 3 days. In one series of experiments, *minimus* pupae in Bengal matured in 108 hours at 16° C (60·8 F) 66 hours at 20 C (68 F) 42 hours at 24 C (75 2 F) and 30 hours at 30 C (86° F) At 35 C (95 F) the pupae failed to become adults. At 11·7 C (53 F) *quadrimaculatus* pupal stages lasted nearly 310 hours and below this temperature there was no development. Anopheline pupae do not hibernate, and they are destroyed by freezing or drying

Larvae are heavier than water but pupae lighter The latter dive by forcing themselves down in a series of somersaults most species rising immediately they become quiet Some species, such as *albimanus* in Panama, pupate usually by day while others like *mediopunctatus* appear to do so mostly at night *Eclosion* or *emergence* of adults from pupae is usually by day and in some species this usually takes place at a given time of day

As emergence time approaches air appears between the now fully-developed *imago* and its enclosing pupal skin giving the pupa a silvery appearance The adult insect escapes through a median dorsal split swallowing air which tends to straighten the abdomen

and to make it more rigid. The process of extrication takes some 2 or 3 minutes, and the insect may then rest on the pupal case or



FIG. 34 Collecting *Anopheles* adults from ceiling of cowshed. World Health Organization team in Pakistan. (*Courtesy World Health Organization, Malaria Section.*)

nearby vegetation for several hours before flying away. During this time the integument becomes hard and dry. The period of emergence is somewhat critical, and such unfavourable factors as wave action or raindrops may result in death of the insect by drowning while entangled in the pupal case.

The Adult

Environment *Anopheles* species also differ greatly in their adult environmental preferences. Some, like *culicifacies* in India, prefer open country; others, such as *umbrosus* in Malaya, are most often in mangrove jungle, or, like *leucosphyrus*, are in forest jungle; still others, like *stephensi* in Bombay, are highly urbanized and live in human habitations. Some, as, for instance, *barianensis* in India, range from 5 000 to 8 000 feet, or, like *gigas baileyi* in Tibet or *pseudopunctipennis* in South America, may range even higher. Others stay mostly in the foothills. *Fluviatilis*, for example, ranges in India between 1 000 and 5 000 feet. Others, like *litoralis* in the Philippines, remain in low seaside areas, or, like *philippinensis* in Bengal, prefer

flat, inland country All species may at times be found in unexpected places, but most seem to have certain well-marked haunts

Resting behaviour So too there are variations in daytime resting habits of anopheline adults although most seem to prefer quiet, shady and slightly humid places Some, like *walkeri* in America, rest on vegetation or like *punctipennis* sometimes in hollow tree-trunks. Others prefer natural, damp earthy places such as the undercuts in stream banks often selected by *minimus flavirostris* in the Philippines. Many species, like *crucians* in the United States are found in such places as culverts and under bridges. Others frequent animal shelters for instance, *atroparvus* in Portugal seems to prefer rabbit hutches, and *messeae* in Italy is common in cowsheds. Houses and outbuildings attract many anophelines. For example *funestus* in Africa goes directly to such a place after emergence, and does not leave until it has fed and has matured its first batch of eggs. Some species, like *quadrimaculatus* in America are found in a variety of places, including tree-trunks culverts stables, under and inside houses. Some species seem to prefer dark and others fairly light resting places e.g *fluviatilis* in India and *superpictus* in Greece respectively Some like *hyrcanus pseudopictus* in India tend to rest low on walls, while others, like *culicifacies* are more apt to be on ceilings or high on walls. Some, like *albimanus* in Puerto Rico appear secretive and are not easily collected in their daytime resting places, which may be on sugar-cane stalks near the ground. Some forest anophelines, like *boliviensis* in Colombia, seem to prefer upper levels and are often in the forest canopy while others, like *gambiae* in Africa, tend to remain at ground levels.

Feeding behaviour Most anopheline adults are quiet by day and active at night. Some, like *bellator* and *cruzi* in Brazil or *atropos* in the United States, will feed day or night. Some species like *culicifacies* in India, are crepuscular as well as nocturnal becoming active in early evening others like *maculatus* in Malaya, are more leisurely and begin their nightly prowling about 9 p m. while still others, like *minimus flavirostris* in the Philippines and *gambiae* in Africa, seem most active after 11 p.m. *Funestus* also in Africa, tends to enter houses between _ a.m. and dawn Some otherwise fully nocturnal anophelines will behave on dark, damp days like diurnal *Aedes* Anopheline females generally take a blood meal the night after emergence but they may delay in cooler weather Some anophelines, like *bellator* in Trinidad will only feed out of doors,

but most vectors feed as a rule inside habitations. Most anophelines feed mainly at ground level, but certain potential yellow fever vectors like *Aedes africanus* usually attack only at tree-top levels.



Fig. 35 Collecting adults of *A. minimus flavirostris* which rest in the daytime in natural shelters along their stream breeding places.

The stimuli attracting a female mosquito to its host include colour and movement, temperature, and moisture, possibly CO_2 tension and, perhaps to a small degree smell. Anophelines seem to bite into black more than white they will not usually attack skin which is below 28 C (82 4 F) and they come much more readily to moist than to dry surfaces. Optimum temperature preference seems to be about 34 C (93 2° F) and relative humidity 70 to 85 per cent. The odours of sweat and of urine are not attractants.

Then there are well marked host preferences among the *Anopheles* species. For example, in the so-called *maculipennis* complex of species in Europe *maculipennis maculipennis messeae* and *subalpinus* are strongly attracted to cattle while *atroparvus labranchiae* and *sacharovi* have a slight preference for man and rabbit, but will go readily to other animals, including cows. *Minimus flavirostris* which is an important malaria vector in the Philippines seems attracted about equally to man and to water buffaloes. But precipitin tests on blood meals of *culicifacies* and *fluviatilis* notable vectors in India, indicate that the former takes blood from cattle some 90 per cent and from man about 10 per cent of the time while these percentages

for *fluviatilis* are almost exactly reversed. Obviousl much higher density of *culicifacies* than of *fluviatil* endemic malaria in a community

Survival The survival of adult anophelines depend ture, humidity genetic characters and natural enemies other factors If the average mean temperature is (95 F) and the average mean relative humidity is u cent or above 90 per cent, it appears to be difficult for to survive Of course they may be able at such times t favourable micro-climates in daytime resting places

Males are shorter lived than females and probably av a few days life in the tropics or during hot seasons elsew they can survive for 1 or 2 months in cooler climates o Females usually live longer but in unfavourable dry seas too may not live much beyond 2 or 3 days perhaps o enough to oviposit once Under favourable conditions man live 6 months or longer Some species like *labranchiae* in S may hibernate through the winter months. The insects fe time to time using the nourishment to replenish their fat rather than to develop eggs except at the end of the hibe period. Hibernating mosquitoes may be able to transmit mal early winter but the oocysts and sporozoites tend to lose infectivity in 6 to 8 weeks after development In Siberia *m* successfully hibernates in cellars where temperatures drop to − 1 (−0·4 F) They become frozen but after gradual thawing are capable of laying the usual post hibernation batch of eggs the south-eastern United States *punctipennis* breeds throughout year and is found in all stages in the winter *crucians* survives cold season mainly in the larval stage *quadrimaculatus* overwint as inseminated females in semi hibernation occasionally tak blood.

In one area different *Anopheles* species may have somewh different seasonal distribution some being most abundant early the season others later For instance in the Balkans *labranchia* tends to be most common in May *sacharovi* in mid-summer and *superpictus* in the early fall. Since each of these species is a malaria vector the effect of the three types of seasonal distribution is to prolong the annual seasonal epidemic in some areas from May to October

Flight The possible flight distance of anophelines is always

longer of course than the *effective flight range* The latter refers to the distance from a breeding place that the females



FIG. 36. A mud-thatched trap for adult *Anopheles*. It is baited at night with an anim

given species travel in numbers sufficient to maintain ende malaria or to cause an epidemic. For instance individual *gamb* mosquitoes may be collected as much as 3 or 4 miles from th breeding places, but generally if *gambiae* larvae are controlled fo distance of only 1 mile (1·6 km.) from the periphery of a tov then malaria transmission will cease within that town *Funestus* l been shown to fly as much as 4 5 miles (7 ˬ km.) but its effect flight range rarely exceeds 2 miles (3 ˬ km) Wind may be important factor in the distribution of adult anophelines. F example, *a prevailing wind may result in most of the output fro* a breeding place flying as a rule in a given direction. In other cas wind may carry anophelines and other mosquitoes many miles in the case of *pharoensis* taken at times by a strong north-east wi for more than 20 miles across absolute desert. Forest or mounta barrier density of a given species in a given area availability ar distribution of food supply for the insect, location of preferred wat for oviposition, genetic characters—all of these and doubtless oth factors may influence flight range. In general, one may say that i the tropics malaria vectors rarely have a greater effective fligh range than 2 miles (3 2 km) and usually not over 1 mile (1·

km) But in temperate zones it is common to find that this range extends for 2 and sometimes for 3 miles (3 2–4 8 km) Local evaluation of the effective flight range is always advisable in planning control operations.

Seasonal flights of anophelines over several miles have been noted For instance prehibernation flights of *sacharovi* in Palestine may cover up to 8 7 miles (14 km) Shorter post-hibernation flights have been noted in the case of *maculipennis freeborni* in America

Swarming and mating In most *Anopheles* species there is swarming of males—a nuptial dance—prior to mating The phenomenon generally does not begin until light intensity is 2·0 ft. candles or less, and the wind velocity something on the order of 200 ft. per minute. The swarming generally ceases after 20 to 30 minutes. It may involve some 50 or less to perhaps 500 or more males. From time to time a female enters the swarm and quickly emerges united to a male The pairs tend to fall to the ground, or to rest on a nearby surface and soon separate

Certain species such as *stephensi stephensi* will mate in a small lantern chimney cage without swarming Other species, like *fluviatilis* require a space of at least 2 ft. by 2 ft. and still other species, like *culicifacies* will not mate unless there is room for male swarming

Not only does the male swarm produce a characteristic humming sound audible to the human ear but the individual mosquitoes have mating calls inaudible to man but which have been magnified several million times and recorded on phonograph discs. The males and females of each species appear to have distinctive tonal emanations which might be useful in classifying doubtful species. The rebroadcast call of a single female will cause the males of the same species to burst into an answering chorus.

Oviposition *Anopheles* females lay their eggs usually at night and, as a rule, singly or in loose groups always on water In some species, as in *multicolor* the female rests on the surface film or on some floating debris while ovipositing Other species, like *culicifacies* oviposit while hovering a foot or less above the water surface Such species will not oviposit where emergent vegetation such as rice plants, are tall enough to interfere with the hovering oviposition dance. Some species touch the water from time to time during oviposition others do not. In many species the females have well-

marked tropisms which send them, with remarkable consistency to characteristic types of water collections to oviposit.

Techniques

General Within the compass of these lectures it is impossible to describe the numerous and interesting techniques whereby *Anopheles* mosquitoes are located in their several stages in nature, are collected and transported to the laboratory are manipulated and colonized, are examined for determination of infection indices of source of blood meal or of age of adult, and how they are mounted in collections Nor will space permit a discussion of the strategy and logistics of research in problems of mosquito behaviour Here it must suffice to make a few general statements and to describe dissection of gut and glands.

Practical field methods include special ways of using cloth sieves to detect mosquito eggs various types of dippers, nets, pie plates,



FIG. 37 The Earle-Magoon portable trap for adult *Anopheles* It is baited at night with an animal such as pony cow or pig

and spoons for gathering mosquito larvae and pupae, suction tubes for collecting adult mosquitoes and diverse gear for transporting

dead and living insects. So too there are traps which are specially designed as attractive daytime resting places or which use light, or animal bait. Some can be attached to windows to catch anophelines entering or leaving a house.

In using such equipment for obtaining mosquitoes one tries always to conform so far as possible to the natural behaviour of the insect. Thus, in dipping for larvae in epiphytic plants, seepages, borrow pits, marshes, or wells and in collecting adults resting on vegetation, under bridges, in houses or in animal sheds, one will use that technique which consistently gives the best results, standardizing the procedures as much as possible. Enthusiasm and perseverance, as well as the ability to see the world from the point of view of the mosquito, are needful attributes. Good maps, well planned collecting stations, adequate sampling, practical records and careful analyses are all important in mosquito surveys, whether for research purposes or for outlining the epidemiology of malaria in an area, or for measurement of mosquito control results. Finally, as more than one malariologist has said, sun-loving anophelines are not effectively sampled by shade-loving personnel, nor can aquatic larvae be adequately scouted by dry-footed collectors.

In the laboratory there are easily learned methods for separating certain closely-related species by means of egg characters. One should also learn how to recognize hairs, scales and veins in order to use taxonomic keys for larvae, pupae, and adults; also how to dissect and recognize the male genitalia in order to identify species otherwise not distinguishable. So too one early learns how to dissect the gut and salivary glands of female mosquitoes and how to recognize malaria oöcysts and sporozoites. (See Figure 49.) Also one learns how to dissect the reproductive system in order to determine approximate age of insect. Finally, the Rice-Barber precipitin test is useful to determine what species of host animal provided the blood meal of a mosquito.

Much has been written about handling and rearing mosquitoes in the laboratory for confirmation of species, for obtaining good specimens to mount, or for experimental use. Insectary methods are now highly developed.

Dissection of mosquito stomach. Dissecting the female mosquito stomach to find oöcysts and sporozoites is neither complicated nor difficult but of course requires practice as well as ability to recognize the plasmodia. The *first step*, having killed the

insect with chloroform, CO_2, pyrethrum spray or some other agent, is to identify and record the species and source of the specimen, with its laboratory or lot number The *second step* is to pull the mosquito's stomach out of the body cavity into a small drop of 0·85 per cent sodium chloride solution on a glass slide. The moves in this step are (*a*) cutting off cleanly the legs and wings (*b*) placing insect on the slide, with abdomen pointing towards observer leg stubs to the left, viewed through a low-power dissecting microscope (*c*) using dissecting needles to nick the insect's outer integument dorsally and ventrally between the fifth and sixth abdominal segments (*d*) holding thorax with one needle-tip while using the other to pull on the end of the abdomen so that the tip separated by the nicks comes off and draws with it the insect's rectum, hind and mid-gut, ovaries, and Malpighian tubules this operation is done gently and the stomach will be loosened from all binding tissues and will almost always break apart in the fore-gut section, just above the stomach (*e*) cutting away the hind-gut with attached rectum and ovaries, removing any section of fore-gut still attached, clearing away the Malpighian tubules which usually tend to obscure the stomach. The *third step* is to examine the stomach for oöcysts. The moves involved are (*a*) transferring the stomach into a fresh drop of saline which has been tinted slightly with methylene blue (*b*) placing a cover slip on the preparation and then warming it very carefully to expand the gases in the gut, thus stretching the wall for easier examination (*c*) making a diagnosis, excluding stomach cells, fat droplets yeast cells, and other artifacts True oöcysts are somewhat opaque, nearly spherical, measure 6 to 80 microns in diameter All but the smallest sizes have a definite cyst wall, often appear to be on a short stock or pedicle and they have dark pigment when less than half-grown. The mature oöcysts are not pigmented but display internal striations which are the sporozoites, clearly recognized when the cysts are ruptured by careful pressure on the cover slip

Dissection of mosquito salivary glands Having completed the gut examination the observer places the insect, head towards him, in another drop of saline. Then, exerting gentle, steady pressure on the thorax with the side of a dissecting needle, employ the other needle to pull the head from the body Usually the salivary glands, affected by the pressure on the thorax, will be pulled out along with and attached to the neck tissues. The gland lobes will

appear as short, finger-shaped structures The glands are cut loose from other tissue, are overlaid by a cover slip and are examined with a high dry lens for sporozoites These appear as spindle-shaped or slightly sickle-shaped bodies within the gland cells or ducts They measure 12 to 14 microns in length Check the diagnosis by rupturing the glands with gentle pressure on the cover slip in order to force some of the sporozoites out into the open

To stain the sporozoites remove the cover slip carefully and being sure that the upper surface is dry invert it upon a small drop of clear balsam in the centre of a glass slide Also save the slid preparation from which the cover slip has been lifted When b th are thoroughly dry they may be stained like blood smears with Giemsa s technique.

Remembering that it is the sporozoites in the salivary gland *at the time of capture* which are being measured in the usual survey ... should not keep mosquitoes in the laboratory in order to allow m ... time for development of the sporozoites They should be dissect... as soon as possible after collection Under experimental conditi... *A quadrimaculatus* has been infected with *P relictum* from ... caught English sparrows. Both oocysts and sporozoites wer... Therefore the possibility of avian plasmodia in *Anopheles* mosq... must be kept in mind, but appears to be slight in most areas

If dissections are being made to provide laboratory mat... may be useful to recall that *P gallinaceum* sporozoites have bee... alive and infective for 24 hours or longer at 25 C (77 F) ... pended in a diluent consisting of saline extract of wash d chi... erythrocytes.

Marking mosquitoes For study of mosquito dispersal th... range migration predation and other behaviour pattern ... are methods of producing radioactively marked mosquit... F r example, fourth instar larvae and pupae can be treated with radioactive phosphorous as $KH_2P^{32}O_4$ at 0·2–0·3 microcuries per ml of water The average radioactivity of the adults emerging from such water will be about 1 44 milliroentgens per hour or some 43 counts per minute. Radioactive strontium Sr^{89} has also been used for this purpose. This method is sometimes an improvement on the usual technique of dusting adult mosquitoes with printer s ink powder of different colours for subsequent recognition

Distribution of Anopheles

In classifying the very wide geographical distribution of anophelines it is customary to use the following six zoögeographical or faunal realms

I *Nearctic Region* from northern Mexico to Greenland and Alaska.

II *Neotropical Region* from central Mexico to southern Argentina including the West Indies

III *Palearctic Region* from the Sahara Desert to northern Europe, including the Cape Verde Canary and Azores islands from northern Arabia to Siberia including all of Iraq Iran, and Afghanistan all the rest of Asia north of 30° N including the Japanese home islands.

IV *Oriental Region* all of Asia not in the Palearctic Region the Indonesian Islands, including the Philippines and Formosa, and as far east as Wallace s Line i.e. to include Roma in the Lesser Sunda group

V *Australasian Region* Australia, Tasmania and New Zealand, New Guinea and all the islands east of Roma in the Lesser Sunda group Micronesia, Polynesia and the Hawaiian Islands

VI *Ethiopian Region* all of Africa south of the Sahara including Madagascar Seychelles Mauritius and Reunion Islands.

Vector Species of Anopheles

The most important malaria vector species of anophelines are listed in Table V while in Table VI are given those species which in certain areas, or under unusual circumstances have been found naturally infected or have been suspected of being local vectors.

In writing the scientific name of a mosquito the genus name (capitalized) and species name (not capitalized, even if a man s name) are given in that order When it is important to be precise, the surname of the one who first named the species is added, without an intervening comma, and then, after a comma, the year of publication If the original account assigned a species to another genus than it now has, then the author s name carries parentheses.

TABLE V

List of the Most Important Vectors of Human Malaria

1. *A. aconitus* Dönitz, 1902
2. *A. albimanus* Wiedemann, 1821
3. *A. albitarsis* Lynch Arribalzaga, 1878
4. *A. annularis* van der Wulp, 1884 = *fuliginosus* Giles, 1900
5. *A. aquasalis* Curry, 1932 = *tarsimaculatus* of authors, in part, possibly *emilianus* Komp, 1941
6. *A. aztecus* Hoffman, 1935
7. *A. barbirostris barbirostris* van der Wulp, 1884
8. *A. bellator* Dyar and Knab, 1906
9. *A. claviger* (Meigen), 1804 = *bifurcatus* (Meigen), 1818
10. *A. cruzi* Dyar and Knab, 1908 *
11. *A. culicifacies* Giles, 1901
12. *A. darlingi* Root, 1926
13. *A. farauti* Laveran, 1902 = *punctulatus moluccensis* Swellengrebel and Swellengrebel de Graaf, 1920
14. *A. fluviatilis* James, 1902 = *listoni* Liston, 1901
15. *A. funestus* Giles, 1900
16. *A. gambiae* Giles, 1902 = *costalis* Giles, 1900
17. *A. gambiae melas* Theobald, 1903
18. *A. hancocki* Edwards, 1929
19. *A. hargreavesi* Evans, 1927
20. *A. hyrcanus nigerrimus* Giles, 1900
21. *A. hyrcanus sinensis* Wiedemann, 1828
22. *A. hyrcanus williamsoni* Baisas and Hu, 1936, probably *hyrcanus* X of Venhuis, 1939 = *A. venhuisi* Bonne-Wepster, 1951
23. *A. jeyporiensis candidiensis* Koidzumi, 1924
24. *A. labranchiae atroparvus* van Thiel, 1927
25. *A. labranchiae labranchiae* Falleroni, 1926
26. *A. leucosphyrus leucosphyrus* Dönitz, 1901
27. *A. maculatus maculatus* Theobald, 1901
28. *A. maculipennis maculipennis* Meigen, 1818 = *typicus* Hackett and Missiroli, 1935
29. *A. maculipennis freeborni* Aitken, 1939
30. *A. messeae* Falleroni, 1926
31. *A. minimus minimus* Theobald, 1901
32. *A. minimus flavirostris* (Ludlow), 1914
33. *A. moucheti* Evans, 1925
34. *A. moucheti nigeriensis* Evans, 1931
35. *A. nili* (Theobald), 1904
36. *A. novumbrosus* (Strickland), 1916
37. *A. pharoensis* Theobald, 1901
38. *A. philippinensis* Ludlow, 1902

* In some areas of Brazil a third Kerteszia—*homunculus*—appears to share in malaria transmission, usually associated with *cruzi*.

39 *A. pseudopunctipennis pseudopunctipennis* Theobald 1901 (Possibly a complex of species.)
40 *A. punctimacula* Dyar and Knab 1906
41 *A. punctulatus* Donitz, 1901 = *punctulatus typicus* Swellengrebel and Rodenwaldt, 1932 also *punctulatus punctulatus* of authors.
42. *A. quadrimaculatus* Say 1824.
43 *A. sacharovi* Favre, 1903 = *elutus* Edwards, 1921
44. *A. sergenti* (Theobald) 1907
45 *A. stephensi stephensi* Liston, 1901
46 *A. sundaicus* (Rodenwaldt) 1926 = saltwater *ludlowi* of authors.
47 *A. superpictus* Grassi, 1899
48 *A. umbrosus* (Theobald) 1903
49 *A. varuna* Iyengar 1924.
50. *A. vestitipennis* Dyar and Knab, 1906

TABLE VI

Anopheles of Secondary or of Doubtful Importance as Malaria Vectors

A. algeriensis Theobald, 1903
Has been found naturally infected in Algeria.

A. amictus amictus Edwards, 1921
Considered an occasional vector in northern Australia.

A. annulipes Walker 1856
Considered an occasional vector in New Guinea and northern Australia.

A. austeni Theobald, 1905
Has been found naturally infected in Angola.

A. bancrofti bancrofti Giles, 1902.
Has been found naturally infected in New Guinea. May be an occasional vector there and in northern Australia.

A. baezai Gater 1933
Has been found naturally infected in Borneo. May also be an occasional vector in Malaya.

A. barbirostris nominata Venhuis, 1939
Occasional vector in Indochina.

A. (Kertesia) boliviensis (Theobald) 1905
Suspected vector in upper Amazon area where it is found in certain bromeliads.

A. brunnipes Theobald, 1910
Has been found naturally infected in Belgian Congo and French West Africa.

A. christyi (Newstead and Carter) 1912.
Found naturally infected in Uganda, with sporozoite index of 4 1 per cent in one area.

A. concolor Edwards, 1938.
Has been found infected in Belgian Congo.

A. coustani coustani Laveran, 1900.
One natural infection reported in Belgian Congo.

A. coustani ziemanni Grünberg, 1902.
Has been found naturally infected in Belgian Congo. Suspected as a vector in Abyssinia.

A. crucians crucians King, 1939
Has been found naturally infected in U.S.A. and Cuba

A. demeilloni Evans, 1933
One natural oöcyst infection reported in Belgian Congo

A. domicolus Edwards, 1916
Has been found naturally infected in French West Africa. (May be conspecific with *A. longipalpis* Theobald, 1903.)

A. dureni Edwards, 1938
Has been found naturally infected in Belgian Congo. (May have been sporozoites of *P. berghei*.)

A. flavicosta Edwards, 1911
Has been found naturally infected in French West Africa and Nigeria.

A. funestus imerinensis Monier and Treillard, 1935
Has been found naturally infected in Madagascar and may have some importance there as vector

A. hatori Koidzumi, 1920.
Has been found naturally infected in Formosa.

A. hectoris Mira, 1921
May be a vector in Guatemala and other parts of Central America tableland.

A. hispaniola Theobald, 1903
Has been found naturally infected in Algeria. Possibly a vector in Transjordan and Syria

A. implexus Theobald, 1903
Has been found naturally infected in Belgian Congo

A. jeyporiensis jeyporiensis James, 1902.
Has been found naturally infected in Eastern India.

A. karwari James, 1903
Has been found naturally infected in Malaya.

A. kochi Dönitz, 1901
Has been found naturally infected in India and Sumatra.

A. letifer Sandosham, 1945
Said to be distinct from *A. umbrosus* Strickland, 1916, and to replace *umbrosus* when jungle is felled. Has been found naturally infected in Malaya and Borneo. Considered important vector by some observers

A. ludlowi toradjala St. ker and Koesoemawinangoen, 194.
Occasional natural infections found in Celebes.

A. lungae Belkin and Schlosser, 1944
Considered to be a vector in parts of New Guinea and the Solomons.

A. maculipalpis Giles, 190_
Has been found naturally infected in French Cameroons.

A. mangyanus Banks, 1906.
Probably a vector in some areas of the Philippines.

A. marshalli gibbinsi Evans, 1935
Has been found naturally infected in Uganda.

A. melanoon subalpinus Hackett and Lewis, 1935
Reputed to be a vector in Tarragona and Valencia, Spain, and in Transcaucasia.

A. michaeli De Meillon and Leeson, 1940
Has been found naturally infected in Northern Rhodesia.

A. multicolor Cambouliu, 1902
Suspected as a vector in Egypt in 1925 on epidemiological evidence.

A. neivai Howard, Dyar and Knab 1917
One infected among 3,000 dissections in Colombia in 1947

A. neomaculipalpis Curry 1931
Found naturally infected in Trinidad in World War II but not considered important.

A. noroestensis Galvão and Lane, 1937
Found naturally infected in Brazil.

A. obscurus Grünberg, 1905
Found naturally infected in Nigeria.

A. oswaldoi metcalfi Galvão and Lane, 1937
Found naturally infected in Brazil but considered unimportant.

A. pallidus Theobald, 1901
Has been found naturally infected in Bengal.

A. paludis Theobald, 1900.
Has been found naturally infected in Belgian Congo and Nigeria.

A. pattoni Christophers, 1926.
Reputed to be a vector in the hills of North China.

A. pessoai Galvão and Lane, 1927
Reputed to be a vector in the Amazon basin.

A. pretoriensis Theobald, 1903
Has been found naturally infected in Transvaal and Southern Rhodesia.

A. punctipennis Say 1823
Has been found naturally infected in the United States.

A. ramsayi Covell, 1927
Has been found naturally infected in Assam.

A. rhodesiensis Theobald, 1901
Found naturally infected in Sierra Leone Suspected vector in Abyssinia.

A. rufipes Gough, 1910
Found naturally infected in Belgian Congo and Northern Nigeria. May be important in French West Africa possibly occasional vector in Sudan.

A. separatus Leicester 1908.
Found naturally infected in Malaya and Borneo

A. splendidus Koidzumi, 1920
Has been found naturally infected in Formosa and Hong Kong

A. squamosus Theobald, 1901
One infection reported in Belgian Congo

A. strodei Root, 1926.
Has been found naturally infected in Brazil.

A. subpictus malayensis Mangkoewinoto 1918
Occasional carrier in Indonesia.

A subpictus subpictus Grassi, 1899.
Has been found naturally infected in Madras and in Indonesia. Reputed to be a vector in Celebes.

A tessellatus Theobald, 1901
Has been found naturally infected in Indonesia, Borneo Indo-China, South China, and Formosa. Reputed to be the vector in Maldive Islands.

A vagus limosus King, 1932.
Has been found naturally infected in Assam.

A vagus vagus Dönitz, 1902
Has been found naturally infected in Indonesia and in Madras.

A walkeri Theobald, 1901
Has been found naturally infected in United States.

A wellcomei Edwards, 1930
One oocyst infection found in the Belgian Congo

Natural History of Vectors

Certain details regarding the principal malaria vectors are included in Table VII It should be noted again that to be an important vector of human malaria the individuals of a species must take human blood repeatedly in those areas in which they are vectors But some are easily deviated to animals and others strongly prefer

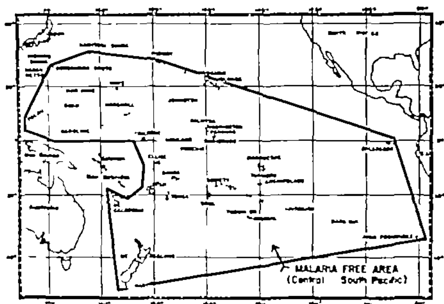


Fig. 38 Malaria free area of Central and South Pacific (Courtesy U S Navy)

human blood. The former become important because of their great abundance or because in the absence of animals they must feed on

A. melanoon subalpinus Hackett and Lewis, 1935
Reputed to be a vector in Tarragona and Valencia, Spain, and in caucasia.

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A. strodei Root, 1926.
Has been found naturally infected in Brazil.

A. subpictus malayensis Mangkoewinoto 1918
Occasional carrier in Indonesia.

A. subpictus subpictus Grassi, 1899.
Has been found naturally infected in Madras and in Indonesia. Reputed to be a vector in Celebes.

A. tessellatus Theobald, 1901
Has been found naturally infected in Indonesia, Borneo, Indo-China, South China, and Formosa. Reputed to be the vector in Maldive Islands.

A. vagus limosus King 1932.
Has been found naturally infected in Assam.

A. vagus vagus Dönitz 1902
Has been found naturally infected in Indonesia and in Madras.

A. walkeri Theobald, 1901
Has been found naturally infected in United States.

A. walravensi Edwards, 1930.
One oocyst infection found in the Belgian Congo

Natural History of Vectors

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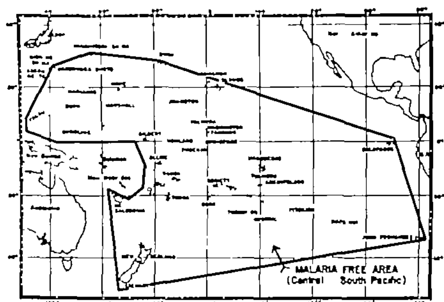


Fig. 38 Malaria free area of Central and South Pacific. (Courtesy U.S Navy)

human blood. The former become important because of their great abundance or because in the absence of animals they must feed on

man They are not so apt to be vectors throughout their geographical range as are the second group But even the mosquitoes in the latter group usually show great variation in their vectorial powers from area to area So the tabular information refers to usual characteristics not invariably applicable since all *Anopheles* species vary habits and habitats from time to time and place to place.

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improvement of land fewer millponds, greatly improved average domestic hygiene, greater use of screens pyrethrum sprays, and

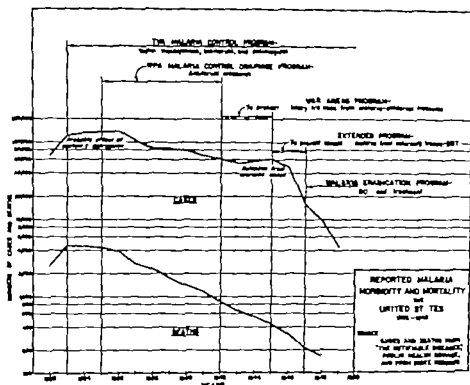


Fig 39 Morbidity and mortality in the United States, 1932–49 (Courtesy CDC, USPHS, Atlanta, Ga.) TVA, Tennessee Valley Authority WPA, Works Progress Administration—a federal relief organization.

malaria remedies, greater density of cattle in the south-east, large exodus of gametocyte carriers into non malarious northern cities, steady development of urbanization in the south-east, intensive use of such malaria control measures as drainage, larviciding space and residual spraying

Intense malaria is still found in areas of the Americas between 15° N and 15 S in Europe south of 45° N in Asia south of 40° N and in Indonesia South-West Pacific, and Africa. In the Central and South Pacific, such islands as the Galapagos Marquesas, Fiji New Caledonia, New Zealand Marshalls, and Carolines are entirely without *Anopheles* mosquitoes. The Hawaiian Islands are also free. On Guam *A subpictus indefinitus* has appeared since the beginning of World War II But to date indigenous malaria has not resulted.

The yearly world total of malaria cases among the estimated

- 38 billions of inhabitants has been the subject of many guesses, none of which of course can be verified. Obviously the disease is rampant in China and equatorial Africa and in parts of India Indo-China, Indonesia and elsewhere One is probably still justified in estimating that the total number of cases of human malaria each year is of the magnitude of 350 000 000 with a mortality rate averaging about 1 per cent. Notable reductions in the not too distant future seem possible in view of the announced plans of the World Health Organization and of a number of governments East and West. But it is incorrect to assume that the newer antimalarial drugs and insecticides have already toppled malaria from the list of important human afflictions however exotic this disease has become in the United States and United Kingdom Perhaps never before in all history have so many human beings been burdened with disease indeed probably three fourths of the world s citizens to-day are without a sanitary environment and among these people malaria still leads the parasitic diseases as a cause of economic, social, and physical disaster

Epidemiology defined How does a malady enter or appear among a group of persons? How does it then spread and persist? What are its cycles and fluctuations its everyday and its exceptional manifestations? How does it recede and disappear? Such are the sort of questions dealt with by the *logos* or science of illness as it rests *epi* or upon the *demos* or people *Epidemiology* in other words, is the sum of what is known about disease as it affects a community rather than a person The physician is primarily concerned with individuals, the *epidemiologist* with aggregations Each has some knowledge and practice in the other s domain but each has unique methods, prescriptions and responsibilities.

The word *endemic* means peculiar to and as regards malaria it connotes the presence of a measurable incidence both of cases and of natural transmission over a succession of years. Malaria is *autochthonous* when contracted locally but is *imported* when infection was acquired outside the specified area Secondary cases derived from those imported are referred to as *introduced* but the natural autochthonous disease is *indigenous* Malaria is *sporadic* when cases are few and scattered and do not follow a pattern of seasonal distribution. The term *malaria endemicity* describes the prevailing frequency and intensity of endemic malaria. The following working classification of the various degrees of malaria endemicity is based

on that proposed by the Expert Malaria Committee of the World Health Organization

1 *Hypoendemic malaria* Spleen rate in children 2 to 9 years of age not exceeding 10 per cent.
2 *Mesoendemic malaria* Spleen rate in children 2 to 9 years of age between 11 and 50 per cent.
3 *Hyperendemic malaria* Spleen rate in children 2 to 9 years of age is constantly over 50 per cent, and the adult spleen rate is also high.
4. *Holoendemic malaria* Spleen rate in children 2 to 9 years of age is constantly over 75 per cent, but the adult spleen rate is low and *adult tolerance is high.*

The word *endemiology* is seldom seen because its restricted meaning is by common usage included within the term epidemiology Occasionally one sees the word *endemy* applied to a disease which is constantly present in an area.

If, in a non malarious area, the disease appears and is naturally transmitted in two successive seasons then in the third and succeeding years of natural transmission it may properly be defined as endemic in the area. In other words imported malaria, with perhaps a few secondary cases the next season is not immediately defined as endemic. Malaria, to be endemic, must persist actively for a period of time, arbitrarily set at three seasons in the above definition Similarly in a previously malarious area if the disease is not naturally transmitted over a period of 3 years, one may then *properly declare that malaria has ceased to be endemic in the* area although some residual splenomegaly and even parasitemia, as well as perhaps the usual density of anopheline vectors, may remain *Potential endemicity may clearly exist, yet malaria may have ceased* to be endemic or on the other hand, may not yet have become so When the various factors which underlie the transmission of malaria *as described below have created conditions thoroughly* conducive to the propagation of the disease, the *endemic potential* is said to be high.

When there *is a considerable and relatively rapid* increase in the incidence of clinical malaria the disease is said to be *epidemic*—a word which connotes a rather abrupt loss of balance. Various types of epidemics are described below Characteristically a state of high *post-epidemic endemicity* remains for a time after an epidemic and is

characterized by relatively high spleen rates and a relatively high degree of immunity among the population concerned

Malaria formula Endemic and epidemic malaria in all of their varied manifestations reflect the complex interplay of many factors The chief actors are obviously man mosquito and plasmodium but there is a large cast of secondary characters Malaria epidemiology is exceedingly intricate but the following formula seems to clarify the subject by focusing attention on the principal variables of which malaria endemicity or epidemicity is a function

Simply stated

$(X + Y + Z)pibect$ = malaria endemicity and epidemicity

In this formula

X	the human carrier	*b*	bionomics
Y	the mosquito vector	*e* =	environment
Z	the human victim	*c*	control
p	the plasmodium	*t* =	treatment
i	immunity		

The X Y and Z constitute the chain of malaria transmission The *p* is the plasmodium factor which by 4 species and diverse strains as already discussed in an earlier section interacts in various ways with the human carrier mosquito vector and human victim The immunity factor *i* in its natural and acquired phases, modifies all three major components of the chain of transmission—mosquito as well as man The *b* stands for the interesting and multifaceted subject of bionomics or natural history not only as it modifies the insect vector and plasmodium but also as it determines those characters and habits of man which intensify or diminish his contact with the mosquito As regards the latter there have already been presented such matters as food preferences, flight capabilities, and various tropisms Bionomics in man or human ecology as it concerns malaria includes such aspects of life as race sex, age occupation economic state agricultural practices, and many others, some of which are described later

The environment is represented by *e* and includes such modifying phenomena as temperature and relative humidity rainfall and topography flora and fauna. The *c* stands for malaria control as described later whether the measures are applied to man or mosquito. Finally the *t* factor is that of clinical and suppressive treatment, applied only to man in practice although there have been

somewhat fanciful suggestions that the plasmodium might be attacked in nature by certain substances imbibed by the mosquito

The human carrier Except possibly as regards *P malariae* in chimpanzees in parts of Central Africa, no known animal reservoir of human malaria exists other than in man himself so that the *source of infection* in human malaria is another infected person Strictly speaking if such an individual *is suffering an acute clinical* attack, he is called a *patient* whereas if his infection is in a latent stage, and he is without symptoms, he is called a *carrier*

A *gametocyte carrier* is anyone in whose blood there are malaria gametocytes. Both male and female mature gametocytes must be accessible in sufficient numbers if a mosquito is to become infectious. Vivax gametocytes develop promptly—within 3 to 5 days of the first appearance of parasitism—but the falciparum crescents are more deliberate so that 10 days usually elapse before a patient becomes a potential carrier Quartan gametocytes are still more dilatory as a rule, and are often scanty In no species is there any constancy about the infectiousness of carriers. Gametocytes appear and disappear without regularity or apparent reason, and there are no gross characters permitting recognition of periods of danger Sometimes children not acutely ill may be highly infectious, specially at ages of 1 to 4 years. The chief seed-bed or source of infection in an area is often located in that segment of the people who are living least hygienically with the poorest treatment of their malaria infections. Naturally the larger the pool of gametocytes the lower need be the density of vector mosquitoes to effect transmission and vice versa.

Malaria may be directly transferred accidentally experimentally or therapeutically by subcutaneous, intramuscular peritoneal, or other parenteral injection of blood, plasma, or other material containing asexual plasmodia or infective sporozoites or certain stages of exoerythrocytic forms Birds and monkeys have been inoculated by oral administration of infectious blood. Intrauterine infection definitely occurs but it is uncommon. Transfusions even when the blood has been stored for 2 weeks at 0 C. (32° F) have caused acute infections, and it is important to realize that malaria may be transmitted by donors who have been symptomless for many years. Quartan malaria, in particular has often been transmitted accidentally by blood transfusion Modern drugs quickly cure trophozoite-induced malaria so that *in an emergency* one should not hesitate to use a potentially malarious donor But ordinarily one

should take precautions to exclude from a donor list all who have ever had quartan malaria or who have had any type of malaria within the previous 5 years.

Mosquito vector The natural history of anophelines as vectors and hosts of malaria constitutes an important part of the malaria formula and has already been discussed Not much more need be included here However one should note the terms *malaria sans anophelism* and *anophelism sans malaria* When malaria occurs in the absence of *Anopheles* mosquitoes it is either imported or residual unless the mosquitoes present have not been found Sometimes there is a short period of transmission in an area and by the time malaria is surveyed the vector has disappeared Another possibility is illustrated by the case of South Ubian a Sulu Sea island where no local anopheline vectors exist The people annually plant, cultivate and harvest their rice on a nearby island where malaria is hyper endemic and where they contract the disease which manifests itself back on their home island

Much more commonly *Anopheles* mosquitoes occur where there is no evidence of malaria. The area may not even be potentially malarious if the local anopheline is strongly zoophilic and rarely takes man s blood In other areas gametocyte carriers are too few or their arrival has been too recent to result in detectable clinical malaria Or there may be too few anopheline vectors. For there is a *per capita critical density* of potential vectors Below a certain incidence of the vector malaria recedes and disappears. The exact figure is difficult to measure and it varies with species and local conditions. For instance in the case of *culicifacies* which usually takes animal blood it requires a high density to propagate malaria A much lower density of a vector like *fluviatilis* is dangerous, because most of the time it feeds on man Moreover a higher *culicifacies* density is required in an area where cattle are abundantly prevalent because there is less need for the insect to feed on man As Ronald Ross noted malaria cannot persist in a community unless the anophelines feeding on man are so numerous that the new infections among the people compensate for the recoveries.

The number of anophelines attracted to a community is proportional to the size of the population but of course the total is limited by output from local breeding places. The number of anophelines per person tends to be in inverse ratio to the size of the community so that malaria risks are often greater in small communities.

table in the Sind of Pakistan has resulted in vastly increasing the density of the pool-breeding vector



FIG. 41 Jungle clearing lets in sunlight and forms favourable breeding place for *A. minimus flavirostris*

Rainfall is of fundamental importance in relation to mosquito breeding but the relationship is sometimes indirect and even paradoxical In the Punjab excessive July and August monsoon rains lead to greatly increased production of *culicifacies* in September but in Ceylon, deficiency in the same monsoon rains will result in certain rivers drying to the point where they become series of countless pools of the type favoured by *culicifacies* In each case an epidemic of fulminant proportions has resulted. In the Philippines the precipitation of the rainy season is often heavy being associated with typhoons so that the quiet stream edges haunted by *flavirostris* larvae are intermittently scoured by raging torrents. But after the rains and before many of the streams have dried this local vector will have increased abundance.

Obviously a 6-inch rainfall in one day differs in its biological effects from the same total precipitation spread over a week, month, or year Some malaria epidemiologists use a formula for *degree of wetness* as follows

$$\frac{\text{No of wet days} \times \text{total rainfall}}{\text{No of days in month}} = \text{Monthly degree of wetness}$$

Temperature and relative humidity ... insect vector as regards its own growth, activity ... well as the *development of the plasmodium* ... observers have concluded that, given suitable relat... range of dry bulb temperature favourable fo... malaria extends from a minimum of about 16·1 C. (61 F ... maximum of about 33·9 C (93 F) A mean 8 a.m. ... relative humidity of not less than 60 per cent seems to be ... for malaria transmission in addition to favourable tempera... requirements These maxima and minima temperature and hum... ity figures are related primarily to longevity of the mosqu... in part to development of the plasmodium, as discussed in an ea... section Of course species requirements and conditions vary ... no fixed and exact averages for optimum conditions can be ...

Temperature effects may be indirect. For instance in t... see Valley average air temperatures above normal in Fe... March may lead to the building up of heavy *quadrim...* tion 4 *months later* Conversely *subnormal spring te...* usually result in lower densities.

Man made malaria Wherever one goes in mal... one sees evidence of man-made nurseries for the larva ... mosquitoes. The list is impressive borrow pits ... villages or to highway railway and canal embankm... and quarry pools drainage obstructed by culverts ... low by fills for rails, roads, and assorted construct... seepage-producing cuts casual siting of villages, ... military encampments careless opening up of ... seeding of a countryside with gametocyte carriers ... drainage or none at all all sorts of man-made wa... like agricultural wells, garden pools eaves ... cisterns, and so on uncontrolled impoundments of ... flood control, or other purpose. Finally there is ... malaria directly due to improper handling and ... water For example, sometimes great quantities ... brought into an area, but there will be no pr... water away Thus in Sind and elsewhere ... water-logging with greatly increased malaria ... irrigation water over-irrigation neglected ... gates, fallow fields unnecessarily wet for prolo... increasing the malaria problem in many places ...—all ar...



Fig. 42. Untidy irrigation created this man-made breeding-place of *A. culicifacies*.



Fig. 43. Wet fallow rice field breeding place of *A. culicifacies*.

Types of Endemic Malaria

Malaria has been classified by climatic types as follows.

Temperate malaria. Found in regions having a severe cold season, with freezing temperatures but with 1 to 3 months of weather suitable for malaria transmission. Mean monthly summer temperatures range from about 15·6° C. (60° F.) to 21·1° C. (70° F.). A spring or summer abnormally cold or hot, dry or wet, may greatly modify the annual malaria incidence. The disease is almost never hyperendemic, it has a tenuous hold, is easily subdued, and has steadily retreated during the past half-century, as in North America and Western Europe. *Plasmodium falciparum* and *P. malariae* cannot usually maintain themselves, and *P. vivax* has the field pretty much to itself.

Subtropical malaria. This type of malaria occurs where mean monthly temperatures are 21·1° C. (70° F.) or more in summer, falling somewhat below 15·6° C. (60° F.) in winter. The latter is relatively mild and short, and includes a well-marked rainy season. Frost and occasional light snowfall are seen, and the adult vectors tend to hibernate or to indulge in periods of no activity. The transmission season is 4 or 5 months long and seasonal epidemics are the rule. *Plasmodium vivax* predominates, but *P. falciparum* is common and *P. malariae* maintains itself in a few foci. Sometimes *P. falciparum* is more abundant than *P. vivax* in the late summer or early autumn.

Tropical malaria. Characteristic of areas where mean monthly temperatures seldom fall below 15·6° C. (60° F.) and maxima at certain seasons often exceed 37·8° C. (100° F.). There are well marked hot-dry and cool-wet seasons. During the former the mean relative humidity may remain for some months below 60 per cent, and during the latter the rainfall may be heavy. Seasonal epidemics of malaria are usual, beginning late in the rainy season and extending until the dry season becomes marked. These epidemics may be severe. Transmission may take place for 6 or 7 months a year. Falciparum malaria is abundant, but vivax predominates in some areas or years. Focal quartan malaria is commonly encountered. Areas of hyperendemic and even holoendemic malaria are characteristic of tropical malaria.

Equatorial malaria. Found where the climate is constantly hot and humid, with heavy, well-distributed rainfall, and with no well-marked wet or dry, summer or winter seasons. Temperatures

rarely exceed 37° C. (98·6° F) or fall below 15·6° C (60 F) Relative humidity seldom drops below 65 per cent, so that malaria transmission may occur at any time of the year Hyperendemicity is the rule, holoendemicity occurs regional epidemics are rare, but localized epidemics common. Vivax malaria is abundant, but falciparum predominates Focal quartan malaria occurs.

Malaria Epidemics

When malaria morbidity or mortality rates or both rise sharply to levels above average endemicity there is said to be an epidemic of malaria in the area affected. Some uncertainty still prevails as to the exact causation of an epidemic. In general one may say that epidemics arise in a given community when there is a relatively large increase in anopheline vector contact with man as well as in the size of the local gametocyte pool, at a time when malaria immunity is low However many factors are involved and the process may be extremely complex. The following *schema* includes the principal points which should be considered in studying the genesis of an epidemic, but, of course, the importance of individual components will vary in different epidemics

There are question marks against some items In the first place, it is not known that fatigue, inanition, or malnutrition will increase a man s susceptibility to malaria but some observers believe so Secondly as regards anopheline susceptibility to infection, it is possible by controlled natural selection in a laboratory to breed colonies of vector anophelines which are highly susceptible or conversely are highly refractory to malaria infection. Does this sort of change ever take place in a given area by an accident of natural selection? So too some individuals of a given species prefer human blood others of the same species take animal blood, the percentages varying for a given species in various areas. Does natural selection by chance sometimes bring about a change in the predominating blood preference of a local anopheline species so that a much larger percentage will feed on man or vice versa, will not? Again some strains of plasmodia seem more prolific in gametocyte production than other strains of the same species, even in the same region. Does this have a bearing on epidemic conditions? Aside from those questions all the other factors named in the *schema* have a proved importance in the causation of epidemics. Used in conjunction with the malaria formula given above the following outline may help to elucidate the genesis of a malaria epidemic

Schema of Points to be Considered in the Genesis of Malaria Epidemics

I INCREASED SUSCEPTIBILITY OF MAN	1 Normal decline in immunity following previous epidemic.
	2. Introduction of non-immunes into an endemic area
	3 Abnormal living conditions causing fatigue, inanition malnutrition (?)
II INCREASED GAMETOCYTE RESERVOIR IN MAN	1 Seasonal relapses.
	2 Lack of treatment.
	3 Importation of human carriers.
	4 An unknown factor leading to increased gametocyte production (?)
III INCREASED CONTACT BETWEEN VECTOR ANOPHELINE AND MAN	1 Increased number of vectors.
	2 Lengthened longevity of vectors due to climatic conditions.
	3 Lessened deviation of vectors from man due for instance, to fewer animals
	4. Decrease in mechanical barriers between man and vector
	5 Greater accessibility of man to vector by change in his habits.
	6 Increased preference of vector for human blood, due to accident of natural selection (?)
IV INCREASED EFFECTIVENESS OF VECTOR HOST	1 Climate more suitable for development of sporozoites.
	2. Increased susceptibility of local anophelines due to accident of natural selection (?)

Some definitions Commonly an *epidemic cycle* of malar consists of 4 periods. One may consider that the first is the *pr epidemic period* a time when the *epidemic potential* is developing- gametocyte reservoir filling local anopheline vector populatio increasing or at least exhibiting a rising infection index. Ne clinical cases are appearing and presently one recognizes the presenc of an epidemic. There next ensues the *epidemic wave period* extend ing from the first notable heightening of incidence of acute cas through the peak of the epidemic curve to that time when morbidit has receded again to normal average endemicity for the localit The curve of case incidence rises more abruptly than it declines, tha of case mortality starts upward about a month after the epidemi wave begins, attains its maximum in a few weeks, and decline rather promptly to the usual endemic level about a month befor the epidemic wave period ends. Spleen index rises with morbidity reaching a maximum 2 months later than the peak of morbidity falling slowly throughout the rest of the epidemic cycle. The para site index climbs more sharply and falls more abruptly than th spleen index. Average parasite counts are suddenly much highe and remain so until the epidemic crest has passed. Frequently ther is increased mosquito vector density which reaches a peak about th same time or a little before the greatest morbidity incidence. Numbers of the insect vector may then shrink rapidly and sometimes fal below normal before the epidemic wave has passed. It is usually bu not always possible to demonstrate an increased density of mosquit vector before or during an epidemic. But, although the number of the insect may not be augmented, yet the infection index in the vector mosquitoes will almost certainly be higher Often both the incidence of the vector and the percentage which are infective will be greater for a time, and this naturally results in a more severe epidemic.

Following the epidemic wave comes a *post-epidemic period* a time of readjustment when the spleen and parasite indices return to the usual endemic levels Then comes the last, or *inter-epidemic period*, which extends to the next pre-epidemic phase. During this quiet interim the spleen and blood indices fluctuate gently around the usual endemic levels and the density of the vector may show only normal seasonal variations But the immunity factor in the human population continues to decline and reaches its lowest point just prior to the next pre-epidemic phase.

There are several types of malaria epidemics. The commonest is

the *seasonal epidemic* generally dependent on increased anopheline production due to seasonal factors which bring about atmospheric and surface water conditions favourable for the development of the insect (and sometimes of the plasmodium within it) Seasonal relapses with increased numbers of gametocytes may also play a part. Yearly epidemics may come in spring summer or fall or in a wet or irrigation season or in the transitional weeks between wet and dry periods Sometimes these epidemics persist for several months sometimes they are very brief Usually the greater the mosquito factor the sharper the epidemic. But this effect may be more complex than it would seem For instance in North Holland as noted above the annual spring epidemic of malaria is usually due to infections which have remained latent since the previous autumn

Plasmodia usually survive seasonal post and inter-epidemic periods in man s blood rather than in mosquitoes. When both *P vivax* and *P falciparum* are involved in seasonal incidence they often have a characteristic relationship to each other Throughout the inter- and pre-epidemic periods, *vivax* is usually encountered more often than *falciparum* During the seasonal epidemic wave vivax malaria has reached its highest point and begun to wane before falciparum malaria arrives at its crest but the latter may eventually be higher than that of vivax

Another common type of malaria outbreak is the *regional epidemic* in which a county state province section or entire country may be involved. These epidemics are most frequent in subtropical and temperate latitudes and they have well-marked periods which tend to be considerably longer than those of the seasonal epidemic cycle. The latter often is submerged but nevertheless, adds its effect in the regional epidemic. The epidemic wave for example, may embrace 2 seasons and the inter-epidemic period may last 10 or even 20 years seldom less than 5 During the inter-epidemic period, seasonal epidemics occur but may be much less severe at first. The pre-epidemic period of regional epidemics may have different characteristics in different areas or times. For example, in Ceylon in 1934 the epidemic potential was greatly augmented by a drought caused by monsoon failure, so that the rivers were transformed into myriads of pools ideal as habitats of the local vector On the other hand in the Punjab of India and Pakistan the pre-epidemic period may be one of monsoon floods in July and August, with excessive pool formation in September leading to an enormous increase in density of the vector In both instances however the pre-epidemic phase

follows an inter-epidemic period during which immunity of the people has fallen to low levels. Sometimes a regional epidemic is due to the introduction of a foreign anopheline vector as in Mauritius in 1867 after the arrival of African *funestus* and *gambiae*

Regional epidemics are sometimes *fulminant* with sudden onset, exceedingly sharp and high rise in case incidence, and a marked increase in mortality For example, in 1906 in the Punjab among a population of 20 million there was a fulminant regional epidemic which resulted in over 300 000 malaria deaths In some areas the mortality rose to 493 per 1 000 Children under 5 years and aged persons were the most common victims

Occasionally regional epidemics spread far beyond the natural boundaries of endemic malaria and then they are called *pandemics* For example, in Russia in 1922–23 a regional malaria epidemic spread as far north as the Arctic Circle and caused the death of millions.

Finally there are *localized epidemics* which arise in unusual circumstances for a given area and are rather sharply circumscribed as a rule. Such outbreaks frequently occur when groups of non-immune emigrants labourers, or soldiers without suitable protection, move into an area of hyperendemic malaria. Entire divisions of troops have been put out of action by such localized epidemics of malaria. Another basic cause of these epidemics has been man made breeding places such as impoundments jungle clearances, and various types of water-holding depressions.

Malaria Surveys

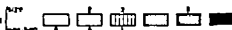
An evaluation of the endemic or epidemic status of malaria in a community or group of individuals is commonly called a *malaria survey* Usually not only prevalence and intensity are estimated, but also such underlying factors as are found in the natural history of the local vectors. Sometimes the term *malaria reconnaissance* is used to indicate a preliminary or brief examination of the malariousness of an area.

In order to differentiate a cyclical fluctuation in malaria endemicity from the decline due to planned control measures the malaria survey frequently covers 3 areas as follows (1) the *protected area* within which the proposed control measures will be or are being applied (2) the *peripheral area* which is contiguous to that protected and by virtue of its nearness shares some of the benefits of the control programme and (3) the *comparison* or *contrast area* which is

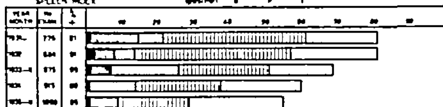
KINGDOM OF ALBANIA DEPARTMENT OF PUBLIC HEALTH

Population 8,000

INDICES FOR VALONA



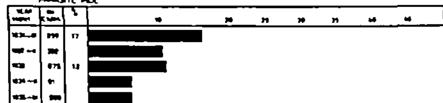
SPLEEN INDEX



DEGREE OF SPLENOMEGALY



PARASITE INDEX



SPECIES OF PLASMODIUM



INFANT MALARIA INDEX

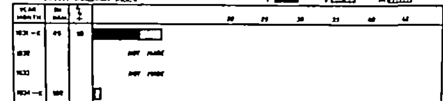


FIG 44 Survey report form used by Hackett in Albania (Courtesy Oxford University Press)

too far away to be affected by the control measures, but is close enough to be subject to the same climatic and other environmental influences. But whether the survey be simple or detailed, the investigator must avoid trying to formulate general conclusions from limited experience. The epidemiology of malaria varies interestingly but complexly from country to country and even from section to section within relatively small areas.

Entire books have been written on the subject of malaria surveys, but the present account must be limited to an annotated outline and some definitions

Annotated Outline of a Malaria Survey

I OBJECTIVE

State specifically Is it for the planning of control measures, for evaluating measures in progress for siting a community for detecting renewed endemicity for pure research, or for what purpose? Is it to be a brief reconnaissance or a detailed investigation?

II. EXISTING DATA

Helpful data may be available in existing medical public health educational, entomological agricultural irrigation, geological, climatological, or other reports or histories. It is often possible to obtain detailed maps even aerial photographs and mosaics Resident officials as well as physicians, may be of assistance in cultivating goodwill but not usually as regards exact details of malaria endemicity

III MALARIOMETRY

A. Malaria morbidity and mortality rates are notoriously inaccurate, but sometimes careful histories obtained house to-house by an experienced observer are useful although not very sensitive indicators of past or present malaria In some surveys it pays to card index all residents by name sex age race, occupation type of house, length of residence protective measures against mosquitoes, history of malarial attacks, use of malaria remedies or of insecticides, and perhaps by additional data regarding size of house in relation to residual spraying procedures

B The simplest and most useful malariometric technique is the determination of the *spleen index* as described below Annual surveys should be comparable as to season sampling observer and

technique If two surveys a year are indicated, one may well be made at the peak the other at the lowest point of seasonal incidence

C. *Parasite indices* should be determined as defined below They are more difficult and more costly to obtain than are spleen indices but usually they are indispensable until the endemicity has receded to a low point. Repeated parasite surveys among infants have special value as regards degree of transmission in a given area

D Frequently it is useful to determine whether average haemoglobin readings in a community or group are normal (13–16 gm per 100 ml. or say 85 to 100 on Tallqvist scale) are subnormal (10 to 12 gm. per 100 ml or say 65 to 80 on Tallqvist scale) or are low (below 10 gm per 100 ml or say 60 or below on Tallqvist scale) This coarse grouping may be all that is required but in research studies more accurate determinations are necessary using photometric technique

IV ANOPHELES VECTOR

Local anopheline vectors must be identified salient features of their natural history recognized and some notion of their larval and adult densities obtained Collections of larvae should be made systematically periodically thoroughly and so far as possible on a quantitative basis. Painstaking surveys are required to discover all the breeding places One is chiefly interested in density frequency resting habitat, blood preferences as determined by precipitin tests sporozoite and oocyst indices longevity and flight range

V PHYSICAL FEATURES

Climatic topographic and any unusual aspects of the environment as it bears on the contact between man and mosquito or on the development of the plasmodium in the insect, should be noted in the survey Impounded water irrigation systems standing water associated with rice dates bananas sugar-cane, or other wet crop agricultural wells local water supplies subsoil water levels behaviour of tides blocked drainage borrow pits brick pits or other man made vectorgenic foci, types of flora and fauna bearing on anopheline oecology jungle and forest growth—these and many other points may be of interest if they contribute to the dissemination, perpetuation or control of malaria So too incoming traffic sometimes has importance because vector anophelines have been known to be imported into an area in carts trucks, trains, boats,

steamers, or aeroplanes They may bring in sporozoites or they may start to colonize an area previously foreign to them.

VI. SURVEY REPORT

Good records including photographs are essential for the survey report. Numerous types of survey cards—some punched for rapid analysis—various display diagrams and many varieties of charts have been suggested for malaria data. Averages or arithmetic means, standard deviation, frequency distribution, probable error or variation, and correlation—all may have a part in analysis of data.

The common standard classification for age groups should be adopted as follows

Age Groups	*Description*
0 to 1	Babies (This group includes infants of ages up to 23 months. The *transmission index* noted below only includes the first 12 months.)
2 to 4	Pre-school children (Toddlers.)
5 to 9	Juveniles. (Sometimes the ages 2 to 9 are combined in a *juvenile spleen index*)
10 to 14	Adolescents.
15 and over	Adults.

The validity of malaria indices will depend on technique and goodness of sample, and the interpretation of the indices will have usefulness in direct proportion to the experience of the interpreter

VII RECOMMENDATIONS

Malaria surveys are usually related to malaria control and will frequently carry recommendations or conclusions of interest to those who must administer public health in a community A survey report, therefore should carry with it a set of well-considered and practical recommendations bearing on the objective.

Spleen Indices

The percentage of palpably enlarged spleens in a group is a *spleen rate* but is an *index* of the endemicity of malaria in the community represented by the group The *spleen index* is generally defined as the percentage of children ages 2 to 14 examined in a given community at a given time, whose spleens on palpation are found to be enlarged. Some observers prefer to use the age group 2 to 9

so in the tabulation of data it is well to keep this age group distinct, and of course one should define the index used An adult spleen index is also sometimes useful in estimating the immune status of a community For instance one of the chief differences between hyperendemicity and holoendemicity is in the fact that the adult spleen rate is high in the former but low in the latter The survey report should note technique used in spleen palpations.

Many ways of classifying degrees of splenic enlargement have been proposed but the following is becoming the generally accepted standard

Class	*Description*
0	A normal spleen not palpable even on deep inspiration
1	Spleens palpable only on deep or at least more than normal inspiration
2	Spleens palpable on normal breathing but not projected below a horizontal line halfway between the costal margin and the umbilicus measured along a line dropped vertically from the left nipple
3	Spleens with lowest palpable point projected more than halfway to the umbilicus but not below a line drawn horizontally through it.
4	Spleens with lowest palpable point below the umbilical level but not projected more than halfway towards a horizontal line through the symphysis pubis.
5	Spleens with lowest palpable point below the lower limit of group 4

The *average enlarged spleen* or *AES* is represented by a number arbitrarily determined by multiplying the number of individuals in each spleen class except class 0 by the spleen class number adding these products and dividing the total by the number of those whose spleens are palpable The resulting figure is the AES which by definition cannot be less than 1·0. An *average spleen* or *AS* may be determined by dividing the total by the number examined i e. including class 0 The *splenometric index* is obtained by multiplying the spleen index by the figure obtained for the AES (See Table VIII.)

Spleen palpation technique If possible the individual being examined should be in a recumbent position head at examiner s left, thighs and legs flexed, entire body relaxed and comfortably disposed The examiner seated at the subject s right, places his hand on the

bare skin in the splenic region at the left costal margin Preferably the area should be exposed but, at any rate, the hand should not be separated from the skin by clothing Various ways can easily be devised to meet the standards of local modesty *Gently* the hand explores the abdomen along the left costal margin, up under it, and down below it, from midline to flank. If no spleen is palpated in the normally breathing subject, the latter is requested to breathe deeply and such forced inspiration may send down a spleen so that it becomes palpable. It is then assigned to class 1 as a palpable-on-inspiration enlarged spleen The larger spleens are classified in accordance with the definitions above. There is also the useful method of Christophers Sinton and Covell of recording spleen size in centimetres of projection below the costal margin But for average purposes the above technique seems highly satisfactory



FIG. 45. Spleen palpation, child standing

If for some reason those to be examined refuse to lie down, or the circumstances will not permit them to almost as good an index can be obtained with the subjects standing The examiner sits or stands at the right with the subject's body facing his right. The examiner's left hand presses the subject's left shoulder forward and downwards while his right hand, on the skin of the left sub-costal region, gently explores for the spleen. If none is found deep inspiration is tried, as explained above The same classification is used. Occasionally as can be demonstrated, a very small spleen will be in class 1 with a child supine, but in class 0 when the child is examined while standing However in practice there is close agreement between the two methods.

Adult splenic indices are not so easily obtained as a rule, because of greater muscular development as well as a greater reticence about the examination. But adult spleen indices have value in the interpretation of immune manifestations among a population group

Therefore, it may be wi[illegible]e to spend the extra time nee[illegible] [illegible] examine adults. Percussion [illegible] sometimes a [illegible] adjunct [illegible] palpation [illegible] locating adult spleens.

The average relationship between [illegible] and para[illegible] in the age group [illegible] to 14 is illustrated by th[illegible] following [illegible] based on the author's experience.

Total examined in series	[illegible]	[illegible]		
Spleen class 0	4,4[illegible] or [illegible]	–	–	[illegible]
" 1	[illegible] or [illegible]		–	[illegible]
" [illegible]	[illegible] or 11[illegible]	–	–	[illegible]
3	542 or [illegible]	–	–	[illegible]
4	[illegible]4 or [illegible]	–	–	[illegible]
" " 5	11[illegible] or [illegible]			[illegible]
Total palpable spleens	[illegible] or [illegible]	–	–	[illegible]

These figures illustrate the fact that from cla[illegible] 1 to cla[illegible] 4 as the spleens become larger the percentage of bl[illegible]d [illegible]mear positives increases. Almost half of the class 1 spleens (49[illegible] per cent) were found in subjects who also had a positive blood smear, a significant figure in view of the doubt which has been expressed ab[illegible]ut the inclusion of this group in a malaria spleen index.

The fact that spleen palpation made to obtain malaria indices requires special training, which general physicians and particularly non-medical technicians do not often possess, requires considerable emphasis. When physicians without malaria survey training work with an experienced malariologist like the late S. T. Darling, there is seldom full agreement about palpable spleens and their classification in any group of children until there has been a period of practice together. After the first or second day, with rare exceptions, there is complete agreement, for there is nothing indefinite about even palpable-on-inspiration spleens. The occasional disagreements after practice will usually be due to success or lack of success in persuading a subject to take a really deep breath. Occasionally a very small spleen may be palpated by a first examiner but not again if the child is tired.

It is surprisingly easy to include a tense muscle or tendon, or the outer end of a rib, with a class 1 spleen, or to diagnose food in the stomach or a hard foecal bolus in the large intestine as an enlarged spleen. One views with great scepticism any spleen rates over 10 per cent obtained in non-malarious communities. Except in the presence of kala azar, exanthematous disease, or a group recently vaccinated against smallpox, the writer has never seen a non-malarious area

bare skin in the splenic region at the left costal margin. Preferably the area should be exposed but, at any rate, the hand should not be separated from the skin by clothing Various ways can easily be devised to meet the standards of local modesty *Gently* the hand explores the abdomen along the left costal margin, up under it, and down below it, from mid line to flank. If no spleen is palpated in the normally breathing subject, the latter is requested to breathe deeply and such forced inspiration may send down a spleen so that it becomes palpable. It is then assigned to class 1 as a palpable-on-inspiration enlarged spleen. The larger spleens are classified in accordance with the definitions above. There is also the useful method of Christophers Sinton and Covell of recording spleen size in centimetres of projection below the costal margin. But for average purposes the above technique seems highly satisfactory


FIG 45 Spleen palpation, child standing.

If for some reason those to be examined refuse to lie down, or the circumstances will not permit them to almost as good an index can be obtained with the subjects standing The examiner sits or stands at the right with the subject's body facing his right. The examiner's left hand presses the subject's left shoulder forward and downwards while his right hand on the skin of the left sub-costal region, gently explores for the spleen. If none is found, deep inspiration is tried, as explained above. The same classification is used. Occasionally as can be demonstrated a very small spleen will be in class 1 with a child supine but in class 0 when the child is examined while standing However in practice there is close agreement between the two methods.

Adult splenic indices are not so easily obtained, as a rule, because of greater muscular development as well as a greater reticence about the examination. But adult spleen indices have value in the interpretation of immune manifestations among a population group

Therefore it may be wise to spend the extra time needed to examin adults. Percussion is sometimes a useful adjunct to palpation f locating adult spleens.

The average relationship between spleen and parasite indices i the age group 2 to 14 is illustrated by the following figures base on the author's experience.

Total examined in group 1 [illegible]		Parasite [illegible] rate
Spleen class 0	[illegible]	[illegible]
1	[illegible]	[illegible]
	[illegible]	[illegible]
3	[illegible]	[illegible]
4	[illegible]	[illegible]
5	[illegible]	[illegible]
Total palpable spleens	[illegible]	[illegible]

These figures illustrate the fact that from class 1 to class 4, as th spleens become larger, the percentage of blood smear positiv increases. Almost half of the class 1 spleens (49.2 per cent) we found in subjects who also had a positive blood smear, a significa figure in view of the doubt which has been expressed about t inclusion of this group in a malaria spleen index.

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Fig 46. Spleen palpation in school house, child lying down.



Fig. 47 Spleen palpation in the field, child standing.

with a spleen index over 10 per cent. He has never been able to find a spleen index above 5 per cent in such non malarious cities as



Fig. 4. Taking blood smears for malaria surveys in Guatemala.

Boston and New York, even taking special care to find the class 1 spleens. Ross and his colleagues never found an index over 1·07 in London school children, Darling never over 1·5 per cent in Fiji, and Boyd not over 2·7 per cent in Lorraine County, Ohio.

Parasite Indices

The percentage of persons examined who have plasmodia in their blood films constitutes the *parasite index*. But of course certain classification is necessary. For instance, it is proper to differentiate *total* or *combined parasite* from, say, a *juvenile* or an *adult parasite index*. Some observers define the *parasite rate* as the percentage of children ages 2 to 14 found at a specified time to have positive blood smears. Other malariologists include only ages 2 to 9. There is also a

transmission index sometimes called an *infant parasite index* which takes in only the ages up to 12 months inclusive. In such a group it *is certain that*, barring a rare congenital case, the infections must have been acquired within the previous year Thus one obtains an index of actual *transmission in a community* Relapses so complicate the findings in older age groups that it may be impossible to gauge correctly the degree of transmission actually taking place.

Commonly thick smears are examined for 5 minutes or thin smears for 15 minutes as a standard practice in determining indices. The number of parasites per cubic millimetre in any given individual is the *parasite count* The mean or average parasite count for a

TABLE VIII

SPLEEN INDICES IN MALARIA

		Town A		Town B		Town C	
Spleen class		*No of children*	*a × b*	*No of children*	*a × d*	*No of children*	*a × f*
(*a*)		(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
0		8	0	7	0	45	0
1		0	0	25	25	4	4
2		22	44	27	54	7	14
3		18	54	1	3	3	9
4		6	24	0	0	1	4
5		8	40	0	0	0	0
Totals	All	62	162	60	82	60	31
	Class 1–5	54	162	53	82	15	31
Spleen index		54 ÷ 62 = 87·1%		53 ÷ 60 = 88·3%		15 ÷ 60 = 25·0%	
AS		162 ÷ 62 = ·6		82 ÷ 60 = 1·4		31 ÷ 60 = 0·5	
AES		162 ÷ 54 = 3·0		82 ÷ 53 = 1·5		31 ÷ 15 = ·1	
Splenometric index		261·3		132·5		52·5	
Interpretation		Intensely epidemic or hyperendemic malaria		Epidemic malaria		Moderately endemic malaria	

community or age group constitutes the *parasite density* and is thus a measure of intensity. Combining counts one can determine *average* and *average positive parasite counts*. *Species infection rates* are often used to determine the *parasite formula* or relative prevalence of species. The average number of parasites present per cubic millimetre of blood of those found positive for parasites is also sometimes called the index of *parasite infestation*. The term *acute infestation* is sometimes applied in a highly endemic area to the early stage of infection among children who have a high parasitemia and acute illness. The term *immune infestation* is then applied to the stage of infection seen in older children in whom parasitemia is low and acute malaria illness much less common.

The *aggregate malaria index*, sometimes called the *Ross index*, is that total percentage of individuals sampled whose blood is found infected with plasmodia or whose spleen is palpable or for whom both examinations were positive.

Mosquito Indices

Anopheles density refers to average number of adult insects of a given species collected per house, per room, per catch, per square foot of resting surface, or per standard trap, or per time period of collecting. It should be defined in each survey report. So too there are various larval density measures as per time dipping, per dip, per square foot of breeding area, or other standard, to be clearly defined in the report. The total number of anophelines in an area constitutes the *Anopheles population*.

The percentage of female *Anopheles* of a given species in which malaria sporozoites are found by dissection is called the *sporozoite index*. An *oocyst index* refers only to oocysts, but an *infection index* combines sporozoite and oocyst findings. Always record the more important sporozoite index separately, whether a combined index is presented or not. The term *infective density* refers to the number of sporozoite-bearing *Anopheles* of a given species per room or house or other area in a given unit of time. The *inoculation rate* is the proportion of a population receiving infective inocula in a given unit of time. Relations between an inoculation rate and a parasite rate are not well understood. Not only are there practical difficulties in measuring mosquito infective densities but the two rates are complicated by the complexity of infection and recovery which

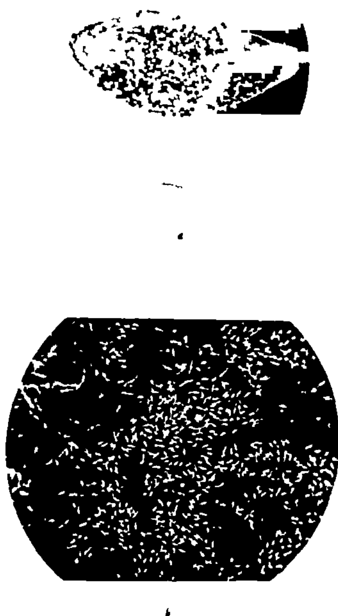


FIG. 49 A, Oöcysts. B, Sporozoites.

in malaria involve such obscure phenomena as immunity, relapse and superinfection. The best clue to amount of malaria transmission in a community comes from a study of infection rates in infants. This is true despite some indication that babies are bitten less frequently than are older children and adults by such a vector as *albimanus* in Jamaica.



Fig 49 A, Oöcysts. B, Sporozoites.

PREVENTION AND CONTROL

Foreword Malaria control is as simple as prescribing 2 tablets of chloroquine a week, and it is as complex as the distribution of residual DDT on the walls of 3 000 000 houses in Brazil in a single year It is as easy as releasing an aerosol spray and it is as technically exacting as the anti-anopheline engineering operations of the Tennessee Valley Authority The old sayings Haste makes waste, and Look before you leap as well as Nothing ventured, nothing gained, all have basic implications as regards malaria control schemes. But the greatest single danger lies in fetishes sometimes cherished by sanitarians who should keep their minds as cleanly functional as their spraying equipment. Beware of what has been called the prejudice of experience. Just now DDT too often receives blind reverence while the study of mosquitoes and of how to eliminate their breeding places is ignored. Yet sound practice involves individual consideration of each local problem to determine the logical solution in the light of available knowledge Drainage and entomological research still have importance in malaria control in certain places Sir Malcolm Watson pioneer in controlling rural malaria by mosquito reduction said There is room for every method in controlling malaria, but from the very first the wise sanitarian will remember that the more economical his operations, the more extensive will be the area he is able to free from the disease.

One should cling tenaciously to sound general principles while departing freely from conventional methods in order to meet local needs. Obviously in view of the complex epidemiology of malaria and the multiple lines of approach to its control those applying funds in this field have a special responsibility to comprehend the significance of what is being done and to determine if it can be done more effectively and cheaply A *practical* understanding of the local epidemiology of malaria, including natural history of vectors, is obviously important, and there should be adequate records and audits of results during and after a project.

For all but the smallest malaria control projects, an organization is required and whether local, state, or national in scope, there should be a staff of trained personnel backed by necessary funds, authority and directives—all on a scale commensurate with the size of the problem.

Classification of control measures A great many measures of malaria prophylaxis and of mosquito control are listed in standard textbooks and one should be acquainted with the literature. For the purposes of these lectures and an average control project, the following list includes the most important.

1 *Measures designed to prevent mosquitoes from feeding on man* using repellents, protective clothing and bed nets screening and site selecting

2 *Measures designed to destroy adult mosquitoes* using sprays and aerosols, both space and residual indoors and outdoors

3 *Measures designed to destroy mosquito larvae* using larvicides intermittent drying sluicing

4 *Measures designed to reduce mosquito density by altering or eliminating breeding places* making naturalistic improvements practising water management filling and draining

5 *Measures designed to combat plasmodia* clinical and suppressive therapy

Pertinent laws and well focused educational measures are often very helpful in facilitating malaria control measures.

Repellents The application of chemicals to skin clothing or bed nets, in order to repel mosquitoes, is a useful measure of malaria control and many substances have been recommended for the pur Ideally a repellent should ward off mosquitoes and other blood icking arthropods for at least 12 hours after application and it should not irritate the skin or have other toxic effects. It should not macerate the skin in hot climates or offend the nose. It should be stainless and harmless to personal effects. It should be easily washed off with soap and cold water but not by perspiration or rain It should be cheap and not subject to deterioration when stored in any climate. Needless to say no such compound is known. But there are four available which have real practicality These are *indalone* (*n*-butyl mesityl oxide oxalate) *Rutgers-612* (2-ethyl-1 3-hexanediol) *dimethyl phthalate* and a mixture of the three called *6-2-2* (20 parts each of the first two and 60 parts of the third) Against *Anopheles* mosquitoes the best is 6-2 2 (average protection, 4 hours) next comes dimethyl phthalate (average 3 hours) Rutgers-612 (average 1 hour) and indalone (average, ¾ hour) In the tropics if one cannot obtain these modern repellents it is usually possible to buy citronella oil which will give protection for periods of 15 to 20 minutes. Renewed at these frequent intervals, it functions well.

In fact, a little citronella added to the repellents named above will increase their usefulness

During the hours when mosquitoes are active, repellents should be applied liberally to exposed or lightly covered skin, avoiding mucous membranes Also avoid wetting any such plastic surface as a watch crystal, fountain-pen case or nylon stockings because the newer repellents are plastic solvents. Anophelines do not always annoy when they feed and therefore they may not be noticeable. Consequently routine application of repellents at dusk and dawn is advisable in malarious areas whether the insects seem to be about or not.

Repellents specially dimethyl phthalate, may be applied to clothing rubbed on by hand, blown on by sprayer or allowed to impregnate as the material is dipped into 15 to 20 per cent solutions or emulsions. The clothing is dried before being worn The impregnation method will impart to clothing a repellency against mosquitoes which may last a week. Bed nets and other netting, even with meshes as large as ¼ in. square (6 35 mm.) when treated with these repellents will turn away mosquitoes for several days.

Protective clothing Special head nets with ¼ in mesh, impregnated with a repellent, may be useful in places where mosquitoes are unusually numerous. Gloves to the elbow and mosquito boots to the knee are also advisable in some areas. Sometimes by wearing 2 or 3 pairs of socks or by using a pillow slip or sarong on avoids the bite of a malaria bearing mosquito on otherwise exposed legs. There are new types of fabric, such as Byrd cloth, nylon-filled poplin, and heavy nylon through which mosquitoes cannot pierce. It is advisable at sunset, where malaria is prevalent, to change from shorts to trousers and to roll down one's sleeves or don a coat. Perversely enough, the hotter and more humid the climate and the less the evening breeze, the more need to use protective clothing nets and screens.

Bed nets When malaria transmission is a nightly possibility specially in rooms unprotected by screening a good mosquito bar or bed net is an effective personal protective measure. Even with screening a bed net is advisable as a secondary line of defence in highly endemic areas. Moreover in a tropical environment, nets often give protection against assorted noxious insects and small verminous creatures.

The mesh of a bed net should be small enough to exclude mos-

quitoes and the thread sufficiently strong to withstand hard usage. Sometimes a finer mesh is used to keep out such small insects as



Fig. 50. Mosquito net for use on bed which has mattress.

sandflies. But all bed nets interfere with air movement and should, therefore, have openings no smaller than necessary. Mosquitoes utilize the greatest, i.e. the diagonal clearance when passing through a cloth or wire mesh opening. Tests indicate that it is physically impossible for a female mosquito to go through an opening smaller than 0·045 in. (1·14 mm.) square (0·064 in. or 1·63 mm. diagonal) and it is generally believed that a 0·047 in. (1·19 mm.) square is safe. The small fibres protruding from the threads of a cloth net make it possible to use wider openings and, if impregnated with a repellent, then a still larger mesh may be tried.

White stiff bobbinet is a suitable material, and rectangular-shaped nets give better protection than do conical. Nets should be reinforced at the corners, have a zippered side opening or none at all, and should be so hung that the body and extremities of the sleeper will not come against the sides of the net. Various modifications are easily made for those who sleep on the floor or in hammocks. The basic idea obviously is to outwit hungry mosquitoes; therefore

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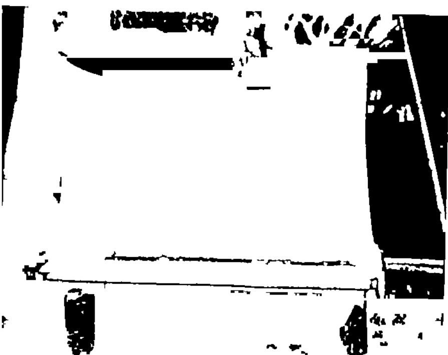


Fig. 50. Mosquito net for use on bed which has mattress.

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possible entrances due to rents in the net or to uneven contact th the floor or bed, are guarded against.



o 51 Mosquito net for use on bed without mattress. This net has tunnels for bamboo s which hold down the edge of the net.

Screening The screening or mosquito-proofing of dwellings s not have the urgency it once had in some formerly malarious fly-ridden areas now protected by residual insecticides. More-r there is still no type of screening which is economically able for the thatched mud hut of rural tropical and subtropical ions. But in many communities screening still has importance inst mosquitoes, house flies, and other insects.

n *properly screened houses, not only are doors and windows* tected but also all other openings through which mosquitoes ght enter The type of wire screening chosen should give quate protection with maximum durability and ventilation. he United States the standard insect wire screening is made of vanized steel commercial bronze, or aluminium, and the standard hes are 16 × 16 18 × 14, and 18 × 18 Wire diameter of the minium type is 0·013 in. and of the other two it is 0·011 in. 16 × 16 mesh, woven wire screening which will exclude most phelines and culicines is generally suitable in the United States.

But it does not keep out all *Aedes aegypti* females, so it is usual in the tropics to specify either 18 × 18 wire screening or else a 16-mesh with a heavy wire Mesh is defined as the width of one opening plus the thickness of one wire Consequently the size of the opening varies with the diameter of wire used in a given mesh size. Any mesh which has openings approximating but not exceeding 0·0475 in square (0·067 in or 1 70 mm diagonal) may be considered as satisfactory in practical use Regular 18 × 18 mesh with a wire diameter of 0·011 to 0·013 in and an average aperture width of 0·0456 in or heavy 16-mesh wire cloth with a wire diameter of 0·0150 in and an aperture width of 0·0475 in are common sizes in copper screening During World War II a new mesh of wire screening was introduced It had 18 warp (length) wires and 14 filler (width) wires to the inch instead of the standard 16 × 16 Hence the opening was rectangular The diagonals actually varied from about 0·0728 to 0·0739 in. It functioned as well as the 16 × 16 but not so effectively as 18 × 18 wire screen

Many materials have been used for screen cloth, including painted steel, galvanized (i e zinc-coated) iron or steel, brass copper aluminium phosphorized bronze monel metal (nickel 67 per cent, copper 28 per cent other metal 5 per cent) and certain plastics The last named will not rust and they are inexpensive but they are easily damaged. Sea air in the tropics is very destructive to screening and it is sound economy to install the most resistant wire cloth available such as that made of aluminium, bronze or of 99 per cent copper In the Canal Zone for many years the standard has been hard-drawn copper wire 99·8 per cent pure 0·015 in. diameter 16 meshes to the inch This gives an aperture of 0 0470 in width Wire screen cloth of all kinds is sold in 100-foot rolls of standard widths varying from 24 to 48 in.

There are some general principles about screening For example screen doors should open outwards not inwards they should be made of seasoned wood and fitted with braces to prevent sagging they require protective panels for pushing or kicking against. Screen doors should close against battens which block crevices between door frame and the wall lintel, or floor and they require a strong spring to ensure automatic tight closing Good workmanship and frequent inspections are desirable In malarious areas, privies should be mosquito-proofed

Site selecting Sometimes a great deal of future woe can be

forestalled if before siting a home, labourers quarters military barracks or encampment, or a new village, one will make an

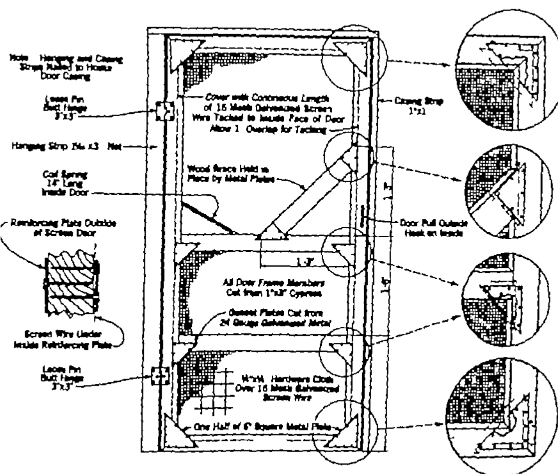


Fig 54. Design and specifications for a screen door (Courtesy TVA.)

appraisal of the local epidemiology of malaria. All too often while scenic or utility features are taken into consideration the potential malariousness of a site is overlooked. Sometimes only a few miles will make all the difference between the need for constant malaria control and for none at all or only a little.

Destroying Mosquitoes

Spraying adult mosquitoes Modern methods for destroying adult mosquitoes consist principally in the use of insecticidal sprays and aerosols (smokes and fogs) indoors or outdoors designed either for immediate effect in spaces or for residual effect on surfaces. In

the past such measures as swatting, collecting, and trapping mosquitoes, burning pyrethrum powder or fumigating with various

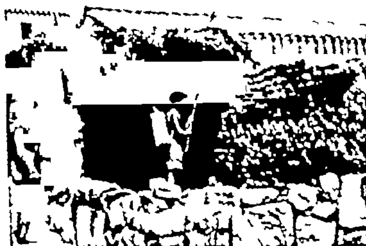


Fig. 53. Residual spraying of a stable in Sardinia

chemicals have been advocated, and more recently the use of dusts of pyrethrum and DDT. But all such methods seem less useful against adult mosquitoes than the latest sprays and aerosols.

The distinction between a *spray* and an *aerosol* is one of droplet size—the former being coarse and the latter microscopic. Aerosols remain suspended in the air for periods up to an hour or more, while ordinary sprays fall rapidly. Insecticidal *fogs*, made up of liquid particles, are wet; *smokes*, consisting of solid particles, are dry. Each is referred to as an aerosol.

Space spraying aims to kill flying or resting mosquitoes by contact with the insecticide at the time of spraying or very soon thereafter, whereas the principle of *residual spraying* is to apply toxicants to surfaces on which insects will rest and from which they can pick up lethal amounts of the toxicant for weeks and even months after the spraying. Of course, space spraying may have some residual effect, and residual spraying may kill those mosquitoes with which it comes into direct contact. Residual spraying is sometimes called *spray painting*, although usually no paint is applied in the process. Both space and residual spraying are sometimes called *spray killing* of mosquitoes.

The term *house spraying* in a community generally indicates that the inside walls or spaces of all the rooms of all the buildings have

been sprayed. *Limited house spraying* implies that only certain structures or only some of the rooms are included in the spraying. On the other hand, *premises spraying* is a term given to the spraying of the inside walls or spaces not only of houses but of outbuildings and animal shelters as well. The words *area spraying* generally indicate out-of-doors spraying of an open space, sometimes an assembly ground sometimes acres of countryside. Both space and residual spraying have been applied to open areas but as a rule there is little residual effect.

In evaluating the malaria control effect of residual spraying one should depend on malariometric indices and not be too much concerned about the incidence of the vector mosquito Any measure which destroys vectors before the malaria plasmodia in them can reach maturity and become infective sporozoites, will interrupt transmission regardless of the prevailing density of the larvae or the numbers of adults being collected inside or outside houses. One effect of residual sprays is to reduce the overall age of the vector mosquitoes so that many do not mature and do not oviposit. Another frequent effect is to reduce markedly the biting rate. Finally over a period of years DDT residual spraying may almost eradicate a vector species but this effect is not a rapid one, so that a single year s spraying may appear not to have had much effect on the insect.

The most sensitive measure of the effects of a spraying project will be the infant parasite or transmission index, frequently reduced to zero by one year s spraying Next, one will note a decrease in intensity of infections as shown by lowered parasite counts later a lowering of the general parasite index, and finally of the spleen index. In highly malarious areas one may see no significant drop in the spleen index for 2 years or more, specially if the sprayed community is not an isolated one. Considerable movement of people in and out of the treated area will delay the appearance of lowered indices. If possible, the area under treatment should be large enough to allow free movement of the people without danger of their becoming infected. Depending on local habits a minimum of 50 square miles may be necessary

Larviciding Prior to and during World War II larviciding was usually the method of choice in killing mosquitoes. But the development of spray killing discussed above, has shifted the emphasis to an attack on adult mosquitoes. In many areas malaria control is

now more economical more certain and more rapid by means of residual spraying and it is not necessary to add larviciding to the



FIG. 54 Using stirrup-pump spraying outfit for residual spraying of DDT in India. (Courtesy Doctor D K Viswanathan, Bombay)

operation. But where for instance a few small river-bed pools constitute the source of the anopheline vector it may be more logical to larvicide these few pools than to spray all the houses. One never says never and always avoids the word always in prescribing malaria control procedures under varying conditions. Both larviciding and drainage which will be discussed later still have usefulness in certain situations.

A perfect larvicide would be strongly poisonous to larvae rapid in action inexpensive fully effective against all species of mosquito eggs, larvae and pupae in all kinds of water easy to formulate to

disperse, and to visualize, non-toxic to all other forms of life—plant or animal. Needless to say there is no such larvicide. Chief reliance



FIG. 55. Larviciding in North Africa with a knapsack sprayer (Courtesy U.S. Signal Corps.)

in the past has been placed in petroleum oils and Paris green. Since World War II several new and useful toxicants have become available. These are discussed below

Mosquito Toxicants

Oils Mosquito larvae have been attacked with oils for many years, but, except as solvents for other toxicants oils have not been used as adulticides. Oils kill larvae chiefly by toxic action exerted directly on the tracheal cells of the insect or indirectly through narcotization, resulting in drowning If the oil blocks the tracheae or is so thick on the water surface that the larvae cannot penetrate the film to obtain air then the larvae will suffocate. But in practice it has been found cheaper and more certain to utilize the toxic effect. *Oil, to be suitable as a mosquito larvicide* should have high toxicity to larvae and pupae, should spread easily and evenly on water surface to form a stable film, penetrate quickly into the tracheal system of larvae, have an inoffensive odour be non-toxic to fishes, waterfowl, and livestock, and be reasonably priced. Such oils are available.

The following specifications apply to good larvicidal oils First the oil should be homogeneous and stable not separating into fractions or depositing solids on standing and it should be free from any foreign material which would clog spray nozzles When applied as a continuous film on clear water it should kill within an hour all eggs larvae, and pupae of all mosquito species it contacts (Certain *Mansonioides* larvae attached to the roots of *Pistia* plants are not contacted by a surface oil film only by emulsions or water solutions. They obtain air through the roots of the plant.) Specific gravity of the oil should be between -7 and 38 viscosity between 30 and 4- initial boiling point between 185 and -30° C. final boiling point, maximum 4-7 C. spreading coefficient minimum 17·0

In open wells or cisterns gasoline (petrol) will kill all aquatic stages of mosquitoes then evaporate leaving no odour or taste in the water It has serious fire and theft hazards. For minor oiling of puddles and peri-domestic drains, kerosene (illuminating paraffin or petroleum oil) may be useful. But its film is thin and easily broken, and the oil is expensive and subject to theft.

Larvicidal oils may be applied by knapsack sprayers various types of power sprayers and from aeroplanes. An active labourer can spray about 5 acres per day with a knapsack sprayer if the terrain is not difficult. The amount of oil required to treat an acre with a uniform larvicidal film will vary from 15 to 20 gals. This requirement is strikingly reduced—to less than a gallon—when the oil contains 5 per cent DDT as described below Various types of oil drip cans and bubblers have been devised for continuous application of larvicide to running water None has proved very satisfactory

Larvicidal oiling has certain disadvantages. For instance, transportation and handling are expensive because of the weight involved water recently oiled is unfit for bathing washing clothes or drinking fishes may be killed by oil wind or rain may disrupt the film soon after oiling oil will not penetrate among grasses or floatage oil repels mosquitoes so that they may deposit their ova elsewhere in untreated, unsuspected water However oil is easily obtainable, it kills all aquatic stages of almost all mosquitoes, and it is plainly visible after application so that inspection is readily carried out. Adding toxicants like DDT greatly adds to the larvicidal effects as described below

Paris green The usefulness of Paris green as an *Anopheles* larvicide was discovered in 1921 by Barber and Hayne. To kill

larvae, this double salt of arsenite and acetate of copper $(CH_3COO)_2$ $Cu\,3Cu\,(AsO_2)_2$ is so applied that small particles of the toxicant will float on water and will be ingested by surface feeding anopheline which will die of arsenic-copper poisoning For such use, Paris green should contain from 50 to 55 per cent of arsenious oxide and be fine enough to pass through a 200 to 300-mesh sieve. Paris green is usually dispersed in the field in the form of a dust mixed with a cheap diluent or carrier such as dry powdered soapstone, talc, slaked lime, road dust, powdered charcoal or some other locally available and satisfactory material.

Paris green larvicidal dusts may be spread by hand, by small bellows blowers knapsack blowers hand or power rotary blowers, and by aeroplane. For hand-spreading 2 per cent Paris green by volume should be used in the dust for small blowers where vegetation is not abundant, 1 per cent may be sufficient. Power blowers, throwing the larvicide for distances up to 200 ft. (61 metres) require 5 to 10 per cent mixtures and for aeroplane dispersal the percentage of Paris green may rise to 50 depending on results of local trials.

The usual dose of toxicant is 10 gm. (0·35 oz.) of pure Paris green per 100 square metres (0·025 acre) of water surface (approx. 14 oz. per acre) or for 80 metres (273 ft.) of bank-side breeding area. Larger doses are required where vegetation is dense. It is generally possible for one man to prepare and spread Paris green larvicide along some 2,000 metres (1 25 miles) of bank per working day This would require 250 gm. (0 55 lb) of Paris green or approximately 25 kg (55 1 lb) of mixture. It is best to spread the Paris green larvicide in the forenoon after the dew has dried and at a time when the larvae are feeding actively

Another method of using Paris green is to prepare a stock suspension having 500 gm. of Paris green per litre (1 1 lb /qt.) of kerosene or Diesel oil. At the place of dispersal, 1 part of this suspension is added to 100 parts of water and applied from a knapsack sprayer

The disadvantages of Paris green are that it is not always easily obtainable, it has no effect on eggs or pupae, and little effect on culicine larvae which feed mostly below the water surface, and the larvicide is not easy to see when making inspections.

On the other hand Paris green is not subject to theft, it is easily transported it does not make the water unfit for bathing washing clothes or even for drinking it does not kill fishes or livestock. Paris green, like all larvicides, is a poison and must be handled

intelligently but in ordinary use it has not been a menace to health.

Paris green has not had any practical usefulness against adult mosquitoes

Pyrethrum The use of pyrethrum powder as an insecticide for household vermin seems to have originated in Persia several centuries ago Introduced into Europe at an unknown date it was first manufactured there in 1828 During the past 25 years, pyrethrum larvicides and particularly imagocides have had considerable value in malaria control At the present time the use of this toxicant is confined very largely to space spraying

The flowers of the plant *Chrysanthemum* (*Pyrethrum*) *cinerariaefolium* grown commercially chiefly in British East Africa, Belgian Congo and Japan contain 0·7 to 3·0 per cent active principles or esters called *pyrethrins* I and II and *cinerins* I and II These are contact poisons to which the cuticle of a mosquito is quickly permeable and to which the insect's nervous system is highly vulnerable These active principles, unstable in the presence of light, moisture and air can be extracted from coarsely ground dried flowers by certain solvents such as kerosene (Somewhat similar insecticidal principles are present in certain other plants. For instance, a substance as toxic as pyrethrins to house flies, which has been identified as N isobutyl-2 6 8 decatrienamide and given the name *scabrin* has been isolated from a Mexican plant, *Heliopsis scabra* Dunal.)

Either as a diluted kerosene extract or as a water emulsion pyrethrum makes an effective mosquito space spray It has a minimum residual effect as ordinarily used but, provided the malaria vector mosquitoes are resting in the shelters at the time of spraying it is possible with pyrethrum sprays to kill them effectively and cheaply By doing this once a week the infected mosquitoes are destroyed before they become infective and malaria transmission therefore ceases, although the density of the vector is not much affected. No doubt the almost universal use of such sprays in the southern United States in the 1930s and 1940s helped materially to reduce the malaria incidence to its present near vanishing point.

Standardized concentrated extracts of pyrethrum are marketed, and when diluted with kerosene they make excellent sprays There are also available many ready prepared pyrethrum sprays for household use Cheaper formulations can be made by simply soaking dried coarsely-ground or crushed pyrethrum flowers in kerosene for 72 hours, at the rate of 1 lb of flowers per gallon of kerosene

A good quality water-white, and nearly odourless kerosene should be used when wall stains and odours must be avoided.

In general the best pyrethrum sprays for killing adult mosquitoes contain no less than 0 4 per cent of the active esters, but commercial sprays average about 0·1 per cent. The dried flowers sometimes deteriorate considerably in storage so that in spraying operations with pyrethrum it is best to test the lots of flowers before use.

Outdoor area space spraying with pyrethrum has successfully protected for several hours gatherings of people occupying from 1 000 sq ft. to 4 or 5 acres. Kerosene 100 gals pyrethrum extract equivalent to 100 lb of flowers, sodium lauryl sulphate 6 lb., and water 50 gals. mixed together make up the so-called New Jersey Mosquito Larvicide. This is diluted with 10 to 15 parts of water and applied for area space spraying as a mist at a concentration of 50 to 150 gals. per acre.

During World War II *liquefied gas aerosols* were extensively used to kill mosquitoes The principal formula was pyrethrins 0·4 per cent (2 per cent of a 20 per cent extract) sesame oil 8·0 per cent, and dichlorodifluoromethane (Freon-12) 90 per cent. As the *liquefied gas is released from a container holding this mixture,* it immediately vaporizes and disperses the pyrethrins into the space sprayed. In other words the Freon-12 acts as a propellant for this so-called *high-pressure aerosol* (67 5 lb /sq in at 68 F increasing to 185 lb at 130° F) A pound of the mixture effectively kills all mosquitoes in .00 000 to 250 000 cu. ft. of space. No less than 40 000 000 1-lb aerosol bombs were purchased by the Armed Forces. Towards the end of the war in order to provide more toxicity to other insects in addition to mosquitoes the formula was modified as follows pyrethrins 0·4 per cent, DDT (aerosol grade) 3 per cent, cyclohexanone 5 per cent, lubricating oil (S.A.E. 10) 5 per cent, and Freon-12 85 per cent.

Low-pressure aerosols have been developed on the same general principle since the war These have pressures of 24 to 40 lb at 70° F There are several formulations. For example, Freon-12 is mixed in equal parts with Freon-11 (trichlorofluoromethane) plus the usual toxicants—DDT and pyrethrins—together with a petroleum distillate and Velsicol AR-50 to keep the DDT in solution. These beer-can aerosols may be sold more cheaply because the lower pressure permits a lighter container

To meet the specifications of a good aerosol, the dispersed particles should not be larger than 50 microns, and at least 80 per

sprayed on surfaces upon which insects walk or rest, as first noted for house flies by Wiesmann in Switzerland in 1942 and for mosquitoes by personnel of the Orlando Laboratory of the U S Bureau of Entomology in 1943 DDT is also a good larvicide.

Essentially *the manufacture of DDT starts with the chlorinating* of alcohol to form chloral alcoholate, which is then broken down into chloral and alcohol by sulphuric acid. The resulting chloral is charged with benzene and oleum in a reactor for some hours to form the DDT which is then washed with water and neutralized with soda ash. *In a dryer the chlorobenzene* is recovered and the DDT is solidified into a white to cream-coloured crystalline solid. The commercial or technical grade is somewhat soft and gummy and consists of a mixture of 70 to 80 per cent para-para prime, and 15 to 25 per cent ortho-para prime isomers and about 5 per cent of some 10 or 12 other compounds The para-para prime isomer is the most important lethal component and the best grades of DDT *should have 80 per cent of this isomer as may be ascertained* by chemical analysis. Recrystallized DDT consists largely of this para para prime isomer

Some observers believe that the trichloromethyl group component of DDT is a lipid solubilizing group and enables the toxicant *to penetrate cuticle whereupon the bis-(p-chlorophenyl) methylene* group kills the insect by attacking vital neuro-muscular centres probably through effects on certain enzymic systems of the insect nervous system.

Solvents DDT is practically insoluble in water but it dissolves *in most organic solvents, which may be classified briefly* as follows (1) *aliphatic hydrocarbons* such as crude oil and petroleum derivatives which have limited capacity for *DDT* (2) *aromatic hydrocarbons* such as velsicols xylene cyclohexanone, and naphthas, which have high DDT capacity (3) *chlorinated hydrocarbons* such as orthodichlorobenzene and trichloroethylene, with high capacity (4) *liquefied gases* such as the freons with low capacity (5) *vegetable oils* such as sesame, castor and cotton seed, with generally low capacity (6) *miscellaneous organic compounds* such as alcohols, ethers, and ketones, with variable capacity (See Table IX.)

Commercial preparations DDT and other new toxicants described below are sold in various oil concentrates, emulsifiable concentrates, dry powders, and wettable powders, as well as in a host of prepared insecticidal spray *solutions or dusting powders*

also as aerosols, described above. The potency of DDT and of other insecticides may be greatly reduced by storage. Therefore they should be used as soon as feasible after manufacture and all lots should have bio-assay tests before use.

TABLE IX

Some DDT Solubilities

Solvent	*DDT Capacity gm./100ml 30° C (approx.)*	*Solvent*	*DDT Capacity gm./100ml 27–30° C (approx.)*
Cyclohexanone	100–120	Acetone	50–58
Methylene chloride	84–91	Carbon tetrachloride	45–48
Benzene	77–83	Ether	29
Velsicol AR 50 (mono and dimethyl naphthalenes)	65–70	Fuel oil (certain Diesel No. 2 oils)	10
		Cotton-seed oil	9–11
Velsicol NR 70 (polymethyl-naphthalenes)	65	Kerosene (crude)	5–8
o-dichlorobenzene	59–71	Kerosene (refined deobase)	2–4
Toluene	60–65	Freons	less than 2
Xylene (commercial)	53	Ethyl alcohol (95 per cent)	1.5

Oil concentrates. An oil concentrate of DDT, or other compound, is simply a concentrated solution of the toxicant in an oil solvent or combination of solvents. DDT concentrates are often 20 per cent, and for ordinary use as residual sprays or larvicides are diluted in kerosene or fuel oil to 5 per cent. DDT is not sufficiently soluble in refined kerosene to permit a 5 per cent spray without an auxiliary solvent, such as xylene or cyclohexanone, often used in making concentrates.

Emulsifiable concentrates A typical DDT emulsifiable concentrate contains DDT 25 per cent (104 5 lb) xylene or other solvent 65 per cent (40·5 gals.) and an emulsifier such as Triton X-155 or X-100 10 per cent (1 gal.) There are many emulsifiers. Chemically Triton X-100 which is typical, is an alkyl aryl poly ethoxy ethanol. The *Brazilian Malaria Service* uses as an emulsifier a 4 to 1 mixture of castor oil and glycerine heated to 200 C. for 2 hours.

Emulsifiable concentrates of DDT or other toxicants are intended for use after mixing with water The one above, diluted 1 part concentrate to 4 parts water will make a 5 per cent finished emulsion. Water-miscible solvents have not proved useful. Volatile solvents like acetone or xylene and non volatile, such as alkylated naphthalenes or a petroleum oil, are satisfactory The *former* evaporate after spraying and leave a deposit of toxicant crystals. The *latter* after the water evaporates, leave the treated surface coated with a toxicant solution. The proportion of emulsifier should be increased when smaller particle size or more permanence of emulsion is required. Emulsion concentrates should mix with water readily and not require special emulsifying equipment, should form suitable emulsions in hard as well as soft, salt as well as sweet waters should be reasonably stable when diluted to concentrations of 0·1 to 10·0 per cent DDT and should be useful for both larviciding and for residual spraying

Dusts or dry powders DDT dusts, and those made with other toxicants may be mixtures of finely-ground toxicant and a diluent such as high-bulk powdered talc, clay or pyrophyllite or a low-bulk diatomaceous earth, or other materials. Insecticidal dusts are also made by impregnating the diluent with a solution of toxicant in a volatile solvent, such as acetone or benzene. Dusts should be so formulated that they have a practical bulk-density ratio and so that they will resist caking on storage at various temperatures. Ready prepared insecticidal dusts generally range from 1 to 25 per cent strength. But as a rule, to overcome the natural gumminess of DDT and to reduce its tendency to lump at least 10 parts of diluent should be added to 1 part of DDT

Wettable powders When a surface active or wetting agent is added to an insecticidal dust, a wettable or water-dispersible powder is formed, and the insecticidal mixture can be suspended in water for dispersal by spraying With certain kaolin types of clay no

Formulation	Summer		Winter (ave temp below 50 F)	
	Power mixer	Hand mixer	Power mixer	Hand mixer
DDT	125 lb	84 lb	70 lb	44 lb
Xylene	31 5 gals.	21 gals.	35 gals.	22 gals.
Triton X 100	2·0 gals.	5½ qts.	7 qts.	5½ qts.

(Formula change in winter is to prevent DDT from crystallizing out at lower temperatures.)

To make a 5 per cent DDT spray containing 50 mg DDT per ml. of spray solution, use 1 part summer concentrate with 6 parts of water or 1 part of winter concentrate with 3 parts of water Apply at rate of 200 mg DDT per sq ft. (approx. 2 1 g/M^2) or 4 ml. of the 5 per cent spray per square foot (1 gal. to 946 sq ft.) The U S Public Health Service estimated the average house in the south-eastern United States to have 2 100 sq ft. of surface to be sprayed requiring 420 gm or 15 oz. of DDT equal to 1 qt. of the summer concentrate and at 4 ml./sq ft. equal to 2 22 gals of 5 per cent DDT spray

To prepare water-wettable sprays one simply adds the water dispersible powder to water being careful to mix thoroughly and to agitate frequently so that the powder will remain in suspension while being sprayed. The sprays are usually made up so that the suspension will contain 10 5 or sometimes 2 5 per cent DDT If the powder is of good quality no particles will be greater than 40 microns 2 5 gm. of the powder will be completely wetted in 50 ml. of water in 2 minutes and at least four-fifths of the DDT will be in suspension after 30 minutes of standing quietly in a standard cylinder The particle size of wettable powder is best at about 35 to 36 microns This powder will pass a 400-mesh sieve. If the inert particles in a mixture are softer than DDT they will become smaller than DDT particles in the grinding process and will tend to coat the DDT and interfere with its toxic action It is best to have the inert particles larger than those of DDT

Thus far the 50 per cent wettable powders have been most suitable. Theoretically it would be better to use a powder containing 75 or even 90 per cent DDT But the 90 per cent powder settles down too rapidly There is a 75 per cent powder which

maintains a suspension for nearly a day but it tends to cake on the second day and is not easily re-suspended

The stickiness of the spray is important. Some formulations adhere better to surfaces than do others. All residual DDT spraying operations should be carefully checked by taking samples of wall scrapings on selected materials from time to time for chemical analysis being careful that the samples are fully representative of the walls sampled

Symes and others have shown that the crystals of toxicant must be of such a weight that the mosquitoes can carry them away If the crystal is large and flat, and if it adheres too closely to the sur face it may not be taken off by the insect. In general crystal size of 10 to 20 *mu* length with average width is best.

Applying Residual Sprays

In applying residual mosquitocidal sprays one aims to coat a surface evenly with a given dose of the toxicant This requires some training For example the fan-shaped spray should strike the wall at right angles from a distance of about 18 in at about 40 lb pressure The hand of the operator should guide the wand or lance at an even speed back and forth with minimum overlap and no gaps in the treated surface If the nozzle is held too close to the wall some of the spray will be deflected and will not stick to the surface face if held too far from the wall much of the material will be blown about as a space spray and will not come into effective contact with the surface to be treated The spray ing should wet the wall but not cause the insect icide to run down in rivulets. Special attention should be paid to those surfaces on which it is known that *the mosquitoes habitually rest.* When a spraying gang is working in a com munity the foreman will do well to keep the workers well clustered together for easy obser vation and not let them scatter all over the landscape

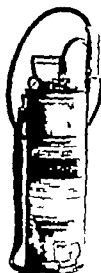


FIG 56. The Lofstrand type of sprayer

Usually there is excellent co-operation on the part of householders in residual spraying oper- ations. But after the novelty and urgency pass, one may encounter refusal rates of from 1 to 10 per cent in rural areas and from 15 to

70 per cent in cities The usual reasons why certain houses cannot be sprayed on schedule are (1) the householder prefers to do the spraying himself (2) he fails to prepare the rooms in time for spraying (3) he finds it too much trouble to get ready (4) he claims there is no need to spray because there are no insects or he has screening (5) he fears damage or toxicity from the spraying

Water-wettable formulations are best on mud or dhobie surfaces, also on reed and cane or rough wood walls These preparations can be used wherever staining of the surface is not contra-indicated. Fine quality water-white kerosene sprays will not stain wallpaper or tinted plaster walls, but such sprays tend to be adsorbed by mud and dhobie to a considerable extent. Surfaces newly coated with an oil paint or enamel also tend to neutralize the toxicant when it is in a kerosene solution

Doses of residual DDT vary from 50 to 250 mg of toxicant per square foot of surface treated. In average usage a dose of 200 mg/sq ft. (2 15 gm./sq metre) is applied. As a general rule, when the transmission season is not over 4 months, one application of 100 mg./sq ft. (1·08 g/sq metre) is sufficient when the season is 5 to 10 months, 200 mg/sq ft. is a more effective dose when transmission is continuous 100 mg/sq ft. 3 times a year is probably best. In countries where the inhabitants are apt frequently to add new coats of mud or whitewash or other material to the inner walls of their huts as in rural India it may be more practical to apply the smaller dose more frequently Local conditions such as labour costs, natural history of vector customs and homes of the people, all require consideration in planning the formulation dosage, and frequency of application. All the houses in a given area should be treated none omitted for reasons of economy

Spraying equipment should be in good working condition before being taken into the field, and should be cleaned and restored to good order at the end of the day Precautions against toxicity to man and domestic animal are given below

Residual DDT may be applied to screening (and, if no sprayers are available, to solid surfaces) by using a paint brush, a felt mop, or other type of applicator

Recently there have been promising experiments with the use of commercial DDT liquefied at about 100 C. and applied thus as a residual spray without solvents, emulsifiers or sticking agents. Certain practical difficulties in regard to field equipment remain to be solved, but the residual coating obtained by this method seems

to be highly effective. On some surfaces the deposit may prove to be objectionable or damaging

Most anopheline species will rest on a DDT-coated surface without being irritated, at least until they have taken up a lethal dose of the toxicant, although they generally show more activity on a treated than on an untreated surface A proportion of individuals of the *A gambiae* species in East and West Africa seem to be unusually sensitive to DDT so that they will not remain on a treated surface. They are not resistant to DDT but they are not killed by DDT residual spraying because they will not tolerate sufficient contact. In certain areas in the Orient, *A minimus* is said to have a similar behaviour There is some evidence that doses of DDT under 100 mg /sq ft are more irritating to *A gambiae* than are larger doses. If such irritation is manifest in only a small percentage of the adults of a vector species in a community it might not make much difference but if any considerable proportion avoid the DDT then another toxicant, such as benzene hexachloride should be used, as described below Interestingly while BHC is said not to irritate *gambiae* or *minimus* it has been reported to irritate *maculatus* in Malaya more strongly than does DDT Neither DDT nor BHC exerts a repellant effect on anophelines seeking to enter a dwelling

It must be emphasized that as stated above if an infected mosquito rests long enough on a DDT treated surface only once during the incubation period of the sporozoite (10 days or longer) then that mosquito dies before it becomes infective Thus it is possible with DDT residual spraying to reduce malaria carried by such mosquitoes as *A. bellator* which rest and feed outside as well as inside houses Enough females do in fact, rest inside for sufficient lengths of time within the incubation period to interrupt the transmission of malaria So too malaria control due to residual DDT has been reported in practice from several areas where *gambiae* is the vector despite the irritating effect of the toxicant noted above.

In certain communities a majority of the people during warm weather sleep in the fields in order to guard their flocks or grain They may or may not use shelters, and they are frequently in almost nightly danger of malaria infection. This obviously prejudices the success of a residual spraying project if it means that the vector mosquitoes do not rest as usual in the buildings which have been sprayed. Under such conditions one should experiment with sprayed traps sprayed nets or sprayed temporary shelters. In some cases it may be necessary to resort to large-scale larviciding if the

population group is great enough to warrant the expenditure. Perhaps suppressive treatment with chloroquine or chlorguanide would be the most logical answer in some areas. If the migrant groups are small, then in the long run residual spraying may solve the problem by its general effect in reducing the parasite reservoir

The costs of DDT residual spraying will vary according to local conditions It averages about $2 69 per spraying of 100 mg /sq ft. per house in the United States. The Pan-American Sanitary Bureau estimates that in Central America it costs from 50 to 60 cents *per capita* per spraying at 2 gm./M² Italian mainland costs average just under 50 cents *per capita* per year In India costs average some 6 3 cents *per capita* of population protected per year

DDT was first used as an outdoor space spray against adult mosquitoes in the fall of 1943 in experiments carried out under the supervision of the Orlando Laboratory of the Bureau of Entomology and Plant Quarantine in Florida. This method has had wide application against pest mosquitoes, some species of which unfortunately are becoming resistant to DDT as noted below

DDT larviciding The use of DDT as a residual spray has overshadowed its worth as a larvicide. Yet there are communities where DDT larviciding is the method of choice in combating malaria. In *one such it was estimated that malaria could be controlled by DDT* larviciding for some 25 years at the cost of one DDT residual spray treatment. A few dry season river pools were the only source of malaria vectors With a little experience and a few hours of investigation one should be able to determine whether or not to recommend larviciding or residual spraying in a given community It usually wastes money to do both.

DDT does not appear to have been tested as a culicine larvicide in Switzerland prior to 1943 It is probable that the first experimenting with DDT against anopheline larvae was at the Orlando Laboratory of the Bureau of Entomology and Plant Quarantine in February 1943 The first use of DDT as a larvicide from an aeroplane was at Pickwick Reservoir and Wilson Dam in July and August, 1943 by personnel of the Health and Safety Department of TVA in co-operation with the U S Bureau of Entomology and Plant Quarantine. Since 1943 DDT has had wide use as a mosquito larvicide, chiefly in 3 forms (1) blown on water as a dust mixture (2) sprayed in an oil solution and (3) sprayed as an emulsion which tends to penetrate beneath the water surface.

DDT is practically insoluble in water it floats indefinitely being non wetting it resists sinking by rains and neither water nor sun seem to have much effect on its stability as a larvicide Signs of DDT poisoning develop more slowly in pupae than in larvae However although pupae are not always killed few adults succeed in emerging and escaping from DDT treated waters. Most are killed during or just after emergence Used as a dust larvicide the dose should be 0·1 lb of active DDT per acre of surface or 1 lb /acre of a 10 per cent dust (approx. 1 kg /hectare) Twice this dosage is better in the presence of considerable vegetation Various types of diluents, such as talc and pyrophyllite are suitable but not lime because it will hasten the deterioration of DDT

For larviciding DDT is most often used in oil solutions generally at the rate of 0·05 lb DDT in 1 gal. of fuel oil per acre (-- 7 gm in 3 8 litres per 0 4 hectare) applied as a fine mist spray Anopheline larvae can be killed with even smaller doses sometimes as low as 0·025 lb per acre Sometimes resin or other spreading agents (e.g Triton B-1956 or equal) at the rate of 0·25 to 0·75 per cent, are added to the oil solvent. Where there is much vegetation the dose of DDT solution may be increased to 0 1 or 0·2 lb per acre, but some fishes will be killed at this dosage

When emulsions are used the same basic dosage is required as with solutions Emulsions may be more effective against culicine larvae than are the dusts and the oil solutions for as noted earlier the culicine larvae are largely sub-surface in contrast to the anophe lines which float close to and parallel with the water surface, feeding at the top But since emulsions tend to permeate throughout water they may kill fishes and other fauna. In treated water any concentration of DDT greater than 1 part per million constitutes a menace to fishes, also to men and animals drinking the water or washing in it. In fact, some observers have noted that DDT in concentrations as low as 1 in 10 million may kill fishes The emulsions are most apt to have undesirable toxic effects and the dusts are least harmful.

By aeroplane treatment, 0·05 to 0 10 lb DDT per acre may be applied as a 20 per cent solution in methylated naphthalenes such as Velsicol NR 70 or Solvacide 544 B

Except under unusual conditions with larger than safe doses DDT larviciding does not have a practical residual effect. In land locked ponds, with fairly stationary levels, DDT emulsion applied at the heavy rate of 3 lb per acre will destroy mosquito larvae for 8 to 16 weeks. The toxicant adheres to the vegetation and floatage

This dosage of DDT kills all fishes and may be a menace to small birds and mammals

There are other ways of using DDT as a larvicide. For example, fine dry sawdust may be mixed with a 2 to 5 per cent DDT oil solution at the rate of about 1 5 qts of oil to 4 lb of sawdust. The treated sawdust is then sprinkled on the breeding place at suitable intervals Finely-ground cork may be used instead of wood sawdust. Various types of DDT briquettes or larvicidal balls have been used with good effect. For instance, DDT-treated sawdust may be incorporated in plaster which after hardening, is thrown into water It gradually disintegrates and frees the toxic sawdust particles which rise to the surface and spread DDT To keep jars and barrels free from *Aedes* larvae 2 per cent DDT in alcohol (ethyl) has been used successfully

DDT Analogs

There are at least 21 analogs of DDT none of which has proved so useful as DDT itself. However mention should be made of (1) *TDE* (DDD or Rothane D3) and (2) the methoxy analog called *methoxychlor* There are also insecticidal *bromine* and *fluorine analogs* (DBrDT and DFDT or Gix)

TDE Dichloro-diphenyl-dichloroethane has about the same toxicity for anopheline larvae but less toxicity for the adults, is more toxic to certain aedines such as *A. dorsalis* and is less toxic to fishes and mammals including man than is DDT It may be formulated and dispersed in the same ways as DDT It has a shorter residual action than does DDT The chief usefulness of TDE is in larviciding projects where fishes have special importance. It is not useful against DDT-resistant flies

Methoxychlor Dimethoxy-diphenyl-trichloroethane may be used in the same formulations and methods of dispersal as DDT but the wettable powder is most useful. However DDT is approximately 10 times more toxic to mosquitoes than is methoxychlor Both have about equal toxicity to fishes Methoxychlor is sometimes effective for a time against DDT-resistant flies. It may be used safely in dairies

Benzene Hexachloride and Lindane

Michael Faraday is said to have prepared benzene hexachloride in 1825 but the discovery of its insecticidal properties was made by

chemists of the Imperial Chemical Industries in England about 1942 The chlorination of benzene in the presence of ultra violet light produces a mixture of isomers of 1 2 3,4 5 6-hexachlorocyclohexane loosely termed *benzene hexachloride* or *BHC* (once called 666) The gamma isomer is the principal insecticidal ingredient and has a trade name of Cammexane. In 1948 the U S Interdepartmental Committee on Pest Control selected the word *lindane* for preparations of the gamma isomer of BHC of a purity of not less than 99 per cent.

BHC has a disagreeable and persistent musty odour which is considerably less pronounced in lindane. Crude BHC should contain 12 to 15 per cent gamma isomer and there are semi-refined BHC products containing from 20 to 75 or 80 per cent gamma isomer In buying this insecticide it is useful to figure costs on the basis of gamma isomer since this is the main lethal component. Both BHC and lindane can be formulated as dusts, wettable powders, oil solutions, and emulsions.

BHC and lindane act as contact and stomach poisons and as fumigants against insects. There is a more rapid lethal action to a toxic dose of the gamma isomer than there is to a toxic dose of DDT and both appear to affect the neuro-muscular system of the insect.

While in general the techniques of applying BHC and lindane as residual sprays and larvicides are the same as already described for DDT some comments are necessary The best dosage for residual spraying seems to be 10 mg of the gamma isomer per square foot (108 mg/M) of surface treated. This application will retain mosquitocidal properties for 3 months and must then be renewed, for due to increased volatility of BHC and lindane there is not the long lasting effect seen with DDT

As noted earlier some vector mosquitoes seem to be hypersensitive to DDT and will not stand on a treated surface long enough to absorb a lethal dose These mosquitoes are not disturbed by BHC or lindane so that under such circumstances the latter formulations are more suitable than DDT even though they must be renewed more frequently It was noted above that some vectors may be hypersensitive to BHC By using window and door traps one can test this point in a given locality to assist in the choice of insecticide. In some areas the odour of BHC is welcomed as a sign of powerful medicine in others it contra-indicates its use.

DDT BHC and lindane are all highly toxic to house flies when first applied as residual sprays and this is an added benefit. In the

laboratory BHC is more toxic to house flies but less toxic to adult mosquitoes than is DDT But there are resistant strains of house fly which tend to replace the original mixed population so that by the third year of spraying neither DDT nor the gamma isomer of BHC are effective.

For larviciding lindane promises to be useful, although it is still expensive A suitable dosage is 0 1 lb lindane/acre (112 gm./hectare) Increased amounts of BHC are required depending on the gamma isomer content of the product used Lindane is much less soluble in oils than is DDT Even 2 per cent solutions in kerosene are difficult to prepare

For aeroplane application as a larvicide the usual formulation is a 2 5 per cent gamma in fuel oil at the rate of 2 qts. per acre to provide a dose of 0·1 lb of gamma/acre

There is a Gammexane dispersible powder (P 520) which has had successful trials as a larvicide. The formulation is made up in the field by mixing the powder with water at the rate of 3 oz. per gallon, thus producing a suspension of 0 12 per cent gamma isomer Using a knapsack sprayer this suspension is applied to the mosquito breeding places in streams pools and seepages, and gives excellent kill within an hour Where vegetation is heavy 4 oz. of the powder may be used per gallon of water

A residual BHC mosquito larvicide may be formulated as follows technical BHC 1 lb (12 per cent gamma) Triton X-155 (or equal) 76 ml. xylene to make 1 gal. Diluted with equal parts water and applied at the rate of 1 lb tech. BHC/acre (i.e. 2 gals. finished emulsion) this gives some residual larviciding effect up to a month without much injury to fishes.

BHC is very toxic to bees, 200 times more so than is DDT BHC is more stable in the presence of ferric iron and at temperatures even up to 120° C than is DDT BHC usually should not be used in fogging machines as it may have severe toxic effects on the operators and others.

Chlordane

Another chlorinated hydrocarbon insecticide of considerable usefulness is *chlordane* which has the formula 1,2,4,5 6 7 8 8-octachloro-4,7-methano-3a 4 7 7a-tetrahydroindane Technical chlordane is a mixture of several compounds. The name, spelled *chlordan* by some, is that of the Interdepartmental Committee on Pest Control. Trade names are Octa Klor 1068 and Velsicol 1068

Chlordane is a dark viscous oily and nearly odourless liquid, insoluble in water but readily soluble in most organic solvents. Even more than DDT BHC and lindane chlordane dehydrohalogenates in the presence of weak alkali to form a product non-toxic to insects. Another point is that free chlorine of chlordane reacts with air to form HCl. This reduces or removes insect knock-down and killing properties. Furthermore the HCl acting as a catalyst induces reactions with iron or tin to form ferric or tin salts. These salts mixed with the chlordane when added to kerosene may form precipitates and clog spray nozzles. They may also tend to induce decomposition of the emulsifiers. None of the chlorinated hydrocarbon insecticides should be formulated with chemicals having an alkaline reaction none should be stored in iron or tin containers and each should have been freshly manufactured at the time of purchase. Chlordane concentrates in particular should not be prepared any longer before use than necessary. Drums lined with a phenolic resin or with aluminium are required for shipment. It is wise to bio-assay each lot before use in a malaria control project.

Chlordane is formulated in dusts wettable powders oil solutions, and oil emulsions, and the general comments made earlier about such formulations and dosages of DDT also apply to chlordane. A satisfactory concentrate can be prepared with 8 gals. of technical chlordane 2 gals. of *Triton X 155 (or equal) and 40 gals. of xylene*. This makes 50 gals. of concentrate with approximately 25 per cent technical chlordane. Mix 1 part with 9 parts of water for a 2 5 per cent finished emulsion.

Chlordane is not quite so effective as DDT as a residual spray against flies and mosquitoes and it does not have so long a residual effect. It has been found useful against DDT-resistant flies, but only until the chlordane resistant strain becomes numerous. As a space spray chlordane has been reported to be a little more effective than DDT against house flies but a little less so against *Aedes* mosquitoes. Chlordane has a strong fumigant action against adult flies and mosquitoes although it is also a stomach and a contact poison. Chlordane is more toxic to man and animals than is DDT. At least one death has been reported as due to contamination with a chlordane concentrate. It should not be applied in dairies.

As a larvicide for anopheline mosquitoes, chlordane in experimental tests has been as effective as, or perhaps slightly better than, DDT.

The insecticide *heptachlor* has been isolated from technical chlor

dane. Other names are Velsicol 104 and Velsicol heptachlor A white crystalline solid, it has the formula 1(or 3a)4,5 6 7 8 8-heptachloro-4,7-methano-3a,4,7 7a-tetrahydroindane. It has had successful experimental use against mosquitoes in the same sort of formulations used with chlordane.

Aldrin and Dieldrin

Aldrin is the official name for an insecticidal product containing not less than 95 per cent of 1,2,3,4,10 10-hexachloro-1,4,4a,5 8 8a-hexahydro-1,4 5 8-dimethanonaphthalene and not more than 5 per cent of insecticidally active related chlorinated hydrocarbons. It is a white crystalline solid. It was formerly known as Compound 118 and has the trade name Octalene

Dieldrin (pronounced dēld-rin) is the official name for an insecticidal product containing not less than 85 per cent of 1,2 3,4,10 10-hexachloro-6 7-epoxy-1,4,4a,5 6 7 8 8a-octahydro-1,4, 5 8-dimethanonaphthalene and not more than 15 per cent of insecticidally active related compounds. It is a white, nearly odourless crystalline solid. It was formerly known as Compound 497 and its trade name is Octalox

Both insecticides are available in emulsifiable concentrates (2 lb /gal.) 25 per cent wettable powders and 2 5 per cent dusts. Dieldrin is also available in a xylene solution Both are insoluble in water but soluble in most organic solvents, dieldrin less so in aliphatic petroleum solvents.

Aldrin because of its volatility has less residual activity than that of chlordane, but dieldrin is comparable to BHC in its lasting effects In its immediate effect against house flies, dieldrin is perhaps 40 times more toxic than DDT Against adult mosquitoes it is equally as effective as DDT but it does not seem to irritate the insect as does DDT Neither aldrin nor dieldrin has an appreciable knock down effect on flies. Both are contact and stomach poisons to insects Aldrin also has a fumigant effect. Strains of house flies resistant to each have been noted.

Dieldrin is effective against adult flies and mosquitoes when used at the rate of 8 lb of 25 per cent wettable powder or 1 gal. of 25 per cent emulsifiable concentrate per 100 gals. of spray formulation. As usually applied such formulations contain 0·2 to 0·25 per cent by weight of dieldrin and the deposit on treated surfaces will range between 10 and 20 mg per square foot. Residues will remain effective for some 12 weeks or about as long as BHC.

Concentrations of aldrin as low as 0·01 ppm and of dieldrin as low as 0·006 ppm. gave 100 per cent mortality of mosquito larvae in 48 hours in preliminary tests Aldrin has good larvicidal properties against *Anopheles* in dilutions ranging from 1 in 50 million to 1 in 200 million parts. Dieldrin has somewhat better larvicidal qualities up to dilutions of 1 in 300 million It is more effective against pupae than is DDT or chlordane Dieldrin larvicide dosages should be one-half to one fourth those of DDT

To fishes aldrin is more toxic than is DDT and dieldrin is even more toxic than aldrin Both must be used with great caution where fish life is important. These products are also toxic to warm-blooded mammals—considerably more so to man than is DDT—and must be handled carefully Neither should be applied in dairies.

It seems likely that aldrin and dieldrin will have usefulness as mosquito larvicides and that dieldrin may be a helpful addition to the list of mosquito residual sprays.

Chlorinated Camphene

One of the newer polychlor insecticides is *toxaphene* or *chlorinated camphene* It is an amber-coloured soft, waxy solid with a mild odour suggestive of both chlorine and camphor It is made by chlorinating camphene to a chlorine content of from 67 to 69 per cent resulting in a compound having the empirical formula $C_{10}H_{10}Cl_8$ but whose structural formula is not certain This insecticide is marketed in the form of solutions emulsions dusts and wettable powders similar to those of chlordane. Toxaphene is readily soluble in the usual organic solvents but is insoluble in water It was formerly sold as Hercules Synthetic 3956

Toxaphene is considerably more toxic than DDT to fishes in fact it is probably as toxic as rotenone Application of 0·125 lb per acre (0·22 ppm.) will kill many species Toxaphene, like chlordane is a liver poison toxic to mammals and it should not be used on pets. The usual insecticide precautions as outlined below should be observed with toxaphene.

Toxaphene, as a residual toxicant at 200 mg/ft² acts more slowly and for a much shorter time than DDT against anopheline adults and is somewhat less active than chlordane. Limited tests indicate that it does not have the irritating and consequently repelling effect DDT exerts on some adult mosquitoes. It is less effective than DDT BHC or chlordane as a space spray As a larvicide in emul-

sion form at 0·1 to 0·2 lb./acre, toxaphene seems to be comparable to DDT but in oil solution it is somewhat less effective than similar formulations of DDT It is inexpensive easily formulated, and may be useful against DDT-resistant larvae of *Aedes* species.

Toxaphene is not at present an insecticide of choice for anti-mosquito operations, but it may come to have usefulness in dealing with species which have become resistant to other toxicants.

Parathion

Parathion is an organic phosphate, with the formula o,o-diethyl-o-p-nitrophenyl-thiophosphate. Synonyms and trade names are E-605 R.B 1018 Compound 3422, Rhodiatox, Thiophos, Niran, and many others Its development stems from chemical war fare research in Germany prior to World War II, and it was unknown elsewhere until 1947 Tetraethyl pyrophosphate, or *TEPP* and hexaethyl tetraphosphate, or *HETP* or Bladan are insecticides closely related to parathion and are even more toxic to man *Gearphos* is a mixture of parathion and its dimethyl analog It, too is very poisonous. Schradan is a similar compound.

Parathion is a dark brown to yellow liquid, soluble in water only to about 20 ppm. and slightly soluble in kerosene and similar oils. It is freely soluble in most aromatic and polar solvents such as alcohol, acetone, and xylene. It has been used experimentally in oil solution emulsion, dry and wettable dusts, and it acts as both a contact and a stomach poison, as well as a fumigant, for flies and mosquitoes adults and larvae. It has been used experimentally in dairies without harm to cattle, and it does not appear in the milk.

Parathion is extremely toxic to man and should not be used in malaria control projects without further experimentation, and always with very great caution.

Precautions in using insecticides All insecticides should be kept out of reach of curious children and near-sighted cooks, and should be handled, mixed, and applied with those basic precautions which all poisons require. Also obviously inflammable formulations should not be sprayed near open fires or stored without due care.

A general idea of the relative toxicities of the insecticides mentioned above can be had from the following table, which lists rough

estimates of probable lethal doses for an adult man Exact information is not available and individuals vary greatly in susceptibility

Toxicant	*Estimated lethal dose for adult man (approximate)*
Methoxychlor	1 lb
DDD	½ lb
BHC (15 per cent gamma)	1 oz
Chlordane	¾ oz.
DDT	½ oz.
Lindane	¼ oz.
Toxaphene	⅛ oz.
Parathion	5 drops
TEPP	3 drops

These insecticides are poisonous when absorbed through any route, including lungs and unbroken skin Any factor favouring absorption accentuates toxicity and vice versa. Oil solutions for example, are more toxic than dusts but some of the dusts as for example of aldrin are far from innocuous. Furthermore chronic may be more important than acute toxicity Knowledge of the toxicity of the newer insecticides to man to wild life and to insects is inadequate Meanwhile one must use special precautions.

Emulsions, solutions, and wettable powder suspensions should not be allowed to remain in contact with skin in fact, all concentrates should be washed off immediately with soap and water This is important. One should always bathe thoroughly and change to clean clothing after working in factory or field or laboratory with insecticides. Food, kitchen and dining-room utensils shelves and tables should be protected from insecticidal sprays. Also cover the baby s bed and the children s toys. Avoid inhalation of insecticidal oil sprays and vapours.

DDT lindane and chlordane used in dairies on cows cattle feed, or watering troughs will result in the appearance of these insecticides in measurable amounts in milk and butter products from the dairies How serious this may be is not known although it has been observed that a man may have as much as 290 ppm. of DDT in his fatty tissues without showing signs or symptoms of toxicity So far as one can determine in spite of tremendous use of DDT there has not been a confirmed death due to the operational use of this toxicant in mosquito control projects.

At routine doses of 0·1 lb/acre, DDT TDE, and chlordane are toxic to fishes and will significantly reduce the population of a pond. At dosages of 0·05 lb/acre DDT is somewhat more toxic than TDE or chlordane. These 3 insecticides appear to have no significant effect on fish populations at dosages of 0·025 lb/acre. Toxaphene is harmful to fishes giving complete kills at 0·2 to 0·1 lb/acre after 2 and 3 applications in deep ponds Kills were obtained at dosages of less than 1 part in 27 million.

The phosphorous compound parathion should rarely be used in mosquito control. It is extremely poisonous and must be handled with great care. Several individuals using this insecticide as an agricultural spray have died of parathion poisoning Protective clothing gloves, and, in closed spaces suitable type gas masks should be worn when handling this dangerous insecticide. If a parathion formulation comes in contact with the skin it should be immediately and thoroughly washed off. If it wets clothing the latter should be removed without delay and the contaminated skin washed carefully

Solvents Xylene, the alkylated naphthalenes, fuel oil, kerosene, and other solvents for the above-named toxicants are systemic poisons when ingested and may also cause severe dermatitis. Therefore, due care should be used in handling the solvents, protecting skin by rubber gloves and eyes by goggles, and avoiding prolonged breathing of the vapours.

Antidotes There are no specific antidotes for poisoning by a chlorinated hydrocarbon insecticide. Of course, the usual emergency treatment with stomach pump or emetic should be instituted when an insecticide has been swallowed. Pentobarbital or phenobarbital medication may control the tremors. Large doses may be required Atropine has been advised for use in parathion poisoning Oxygen and even artificial respiration may be needed. Morphine, ephedrine, and oil laxatives are contra indicated in insecticide poisoning

Insect resistance to toxicants *Musca domestica Culex pipiens* and *tarsalis Aedes communis dorsalis nigromaculis punctor sollicitans taeniorhynchus* and *vexans* have all been reported to have developed resistance to DDT *Musca domestica* has also developed resistance in the field to BHC lindane, and chlordane. Thus far no *Anopheles* species has been reported as showing important resistance to any mosquitocide in the field. Laboratory studies have indicated that it

is possible by selective breeding to develop some DDT resistance in *A. quadrimaculatus* but it is not of the order of house fly resistance and thus far has been unimportant.

Susceptible house flies require only about [illegible]0 micrograms of DDT per gram of female fly to produce a median mortality in [illegible]4 hours but the resistant flies require up to 18,000 micrograms to produce the same result. Both resistant and non-resistant flies readily absorb DDT but the resistant flies rapidly metabolize DDT into innocuous metabolites. Susceptible flies are unable to do this. All strains of DDT resistant house flies retain their tolerance when no longer subject to DDT even to 30 generations. The tolerance seems to be a multiple-gene character which causes physiological and perhaps some morphological changes. There is sometimes cross tolerance to other toxicants.

Vigilance is needed to discover the onset of resistance of mosquitoes and flies to DDT so that other toxicants can be used before the tolerance becomes marked. It is quite possible that *Anopheles* species will appear in the list of resistant insects in due time.

Spraying equipment. A wide variety of apparatus is available for dispersing insecticidal formulations for larviciding and for space and residual adult spray-killing. Money and time can be saved and efficiency gained by making the correct choice of spraying equipment for a given project. (See 3rd report WHO Expert Insecticides Committee 1951.)

The simplest sprayer is the common household atomizer of ½ pt. to 1 qt. (¼ to 1 lit.) capacity, in which a plunger in a metal cylinder compresses air, driving it at right angles across the upper end of a capillary tube which extends from the bottom of the container. The air stream causes the insecticide to be sucked up the tube and then atomizes it into a spray often of rather coarse droplets. Best results against flies and mosquitoes are obtained if the capillary feed tube has an inside diameter of 0·015 to 0·025 in. (38–64 mm.) and the air vent 0·040 in. (102 mm.)

For DDT oiling of minor pools there is an Eagle No. 66 pump oiler ("pistol oiler") with a capacity of only 5½ oz. (163 ml.) which is convenient and easy to use.

Many makes and designs of knapsack and tank-type sprayers exist, with capacities between 2 and 5 gals. (7·6–19 lits.) In these a piston air pump puts 60 to 200 lb. pressure on the liquid forcing it through a spray nozzle. Knapsack sprayers are carried on the

back of the operator He wields a wand or lance connecte end to the spray tank by an oil-resistant hose and having at t end a suitable nozzle and shut-off valve. Some sprayers are continuously others of the compressed air type have storage tanks and are pumped or otherwise given rene pressure at suitable intervals. Paint-sprayers, instead of th wand and nozzle, are more useful for dispersing pyrethru sprays But for residual sprays a nozzle such as the Teej which delivers 0 2 gals (750 ml.) per minute, at 40 lb (2 8 kg/cm.²) pressure, with an 80° spray angle, is suitabl nozzle added to a Lofstrand (Trapido model) outfit m excellent combination for residually spraying water v formulations The Hudson and the Smith sprayers have al widely used

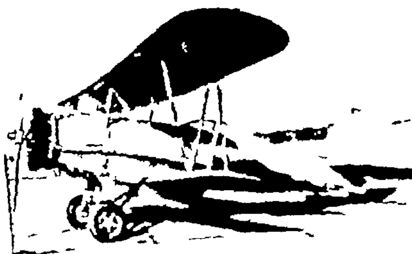
Some sprayers are equipped with 2 tanks, one for liquid a for compressed air These are designed for dispersing the ins at a constant pressure of 40 lb In the Knipe sprayer dry ice fied carbon dioxide) is used as a source of pressure. As the evaporates a pressure of 15 to 18 lb is generated. There small pocket sprayers designed like certain bicycle pumps, th being held in the inner tube or plunger

Another type of sprayer is the stirrup pump which is sim inexpensive and may often be manufactured locally Or pumps continuously while another man sprays. It does not quality work, but may nevertheless meet the practical n some situations. (See Figure 54.)

Spray pump metals, gaskets, and tubing should be m *chemical resisting materials* Seamless construction is ideal tank which is best if made to hold 4 gals. (15 14 lits.) empty a good tank will not weigh over 12 lb (5 44 kg) a accessories not over 8 lb (3 66 kg) There are usually 2 filte through which the liquid is poured into the tank, and the either in the nozzle assembly or at the bottom or base end discharge pipe. The latter type of second filter is useful water-wettable formulations are being sprayed. The delive from a good sprayer is some 500–750 ml./min. (0·13–0·20 ga pressure of about 2 8 kg/cm.² (40 lb/sq in.) The Expert mittee on Insecticides of the World Health Organization second report prepared useful specifications for knapsack, han stirrup pumps.

Hand dusters include small sifter cans, containers with

bulbs, and var ... rotary bl...



Power-driven sprayer ... devised for truck trail ... They vary from small ... gallons and several hundred ...

Liquefied gas aerosol has ... of producing aerosol are (1) ... containing insecticides ... to form a vapour which condenses ... with the air (3) heating a mixture ... water becomes superheated steam ... nozzle breaks up the insecticide ...

Aerosol-generating and thermal ... vehicles or planes some utilizing the ex... devised and are available commercially ... condition for outdoor distribution ... inversion i.e. when the temperature at ... 6-in. level and the insecticide cloud ... ground. Usually 4 or 5 hours after midn... rule because of the absence of much breeze. A ... overcast daytime sky with inversion relatively ... wind movement not over 8 miles an hour ... conditions.

back of the operator He wields a wand or lance connected at one end to the spray tank by an oil-resistant hose and having at the other end a suitable nozzle and shut-off valve. Some sprayers are pumped continuously others of the compressed air type have pressure storage tanks and are pumped or otherwise given renewed air pressure at suitable intervals. Paint-sprayers instead of the usual wand and nozzle, are more useful for dispersing pyrethrum space sprays. But for residual sprays a nozzle such as the Teejet 8002, which delivers 0 2 gals. (750 ml) per minute at 40 lb sq in. (2 8 kg/cm²) pressure, with an 80 spray angle, is suitable. This nozzle added to a Lofstrand (Trapido model) outfit makes an excellent combination for residually spraying water wettable formulations The Hudson and the Smith sprayers have also been widely used

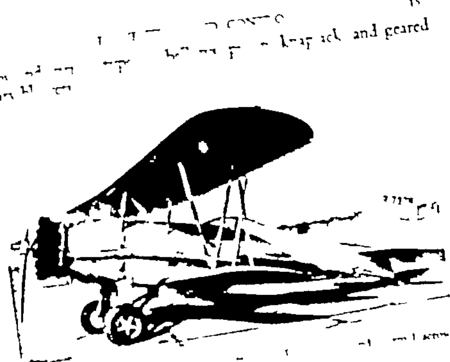
Some sprayers are equipped with 2 tanks one for liquid and one for compressed air These are designed for dispersing the insecticide at a constant pressure of 40 lb In the Knipe sprayer dry ice (solidified carbon dioxide) is used as a source of pressure. As the dry ice evaporates a pressure of 15 to 18 lb is generated. There are also small pocket sprayers designed like certain bicycle pumps, the spray being held in the inner tube or plunger

Another type of sprayer is the stirrup pump which is simple and inexpensive and may often be manufactured locally One man pumps continuously while another man sprays. It does not do high quality work, but may nevertheless meet the practical needs in some situations. (See Figure 54.)

Spray pump metals gaskets, and tubing should be made of *chemical resisting materials* Seamless construction is ideal for the tank, which is best if made to hold 4 gals. (15 14 lits.) When empty a good tank will not weigh over 12 lb (5 44 kg) and the accessories not over 8 lb (3 66 kg) There are usually 2 filters one through which the liquid is poured into the tank, and the other either in the nozzle assembly or at the bottom or base end of the discharge pipe. The latter type of second filter is useful when water-wettable formulations are being sprayed. The delivery rate from a good sprayer is some 500–750 ml./min. (0 13–0·20 gal.) at a pressure of about 2 8 kg/cm² (40 lb/sq in.) The Expert Committee on Insecticides of the World Health Organization in its second report prepared useful specifications for knapsack, hand, and stirrup pumps.

Hand dusters include small sifter cans containers with rubber

knapsack and geared



have been

Power-driven ... and motor boats devised for trucks, trailers ... hundreds of ... They vary from small outfits ... gallons and several hundred ... Other methods ... material

Liquefied gas ... surface of producing ... contact containing insecticides ... to form a vapor which condenses ... water and oil solution until with the air; (3) heating ... when released through a water becomes superheated ... -sized droplets. nozzle breaks up the insecticide ... mounted on motor

Aerosol-generating and thermal ... apparatus mounted on motor vehicles or planes, some utilizing the exhaust engine gases, have been devised and are available commercially. The best meteorological condition for outdoor distribution of fogs and fine sprays is one of *inversion*, i.e., when the temperature at 6 ft. is higher than at the 6-in. level and the insecticide cloud therefore is held near the ground. Usually 4 or 5 hours after midnight is the best time as a rule because of the absence of much breeze. A clear night or an overcast daytime sky with inversion, relatively high humidity, and wind movement not over 8 miles an hour constitute the best conditions.

When considering aircraft dispersal of insecticides one must pay due attention to the following factors initial costs, maintenance

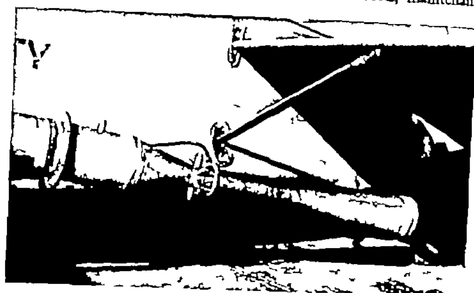


FIG. 58. Closer view of venturi section of exhaust generator (Courtesy TVA.)

costs availability of repair parts and service, operating costs, aeroplane characteristics of load capacity dependability speed range (working and ferrying) range in miles manoeuvrability ease of handling visibility safety down draft. Also one must determine the most suitable formulation size and location of nozzles, speed of plane, control from ground microclimate which influences movement of spray cloud. Distribution of an insecticide from aeroplanes requires skilful piloting Accurate guidance is required either from the ground or from an observation plane. Flags smoke, or captive balloons used as markers, as well as ground-to-plane or inter plane radio communicators are useful to guide the dusting pilot. In some areas helicopters have special usefulness.

Disinsectization of aircraft Many kinds of insects of agricultural as well as medical importance, enter aircraft and may survive long journeys There is undoubtedly danger of inadvertent transport of mosquitoes including those of malaria vector species, from one area to another by air travel. Spraying is the best method of killing insects on planes An insecticide suitable for such use should be rapidly toxic to the insects but non-toxic to man should have great diffusibility be easily applied with simple technique, constitute no fire risk even when engines are running be relatively unobjectionable to passengers, and not damaging to equipment or plastic

windows. No ideal formulation has been devised but an excellent spray is an aerosol containing:

Pyrethrum	1 per cent by weight (i.e. 5 per cent of a 20 per cent pyrethrum extract)
DDT	3 per cent by weight
Cyclohexanone	5 „
Mineral oil	[illegible] „ „
Freon 12	85 „ „

This is sprayed at the rate of 50 mg. of pyrethrins per 1,000 cub. ft. dispersed in 4 to 5 seconds. If no DDT is included the rate of spraying is generally 100 mg. of pyrethrins per 1,000 cub. ft. A variation of this formula reduces the pyrethrum content to 0.4 per cent w/v with application at the rate of 10 gm. of the formulation (i.e. 300 mg. DDT and 40 mg. pyrethrum) per 1,000 cub. ft. or 28 cub. metres.

Baggage and freight compartments may be sprayed before sealing them and the undercarriage before starting the motors. Cabins, if not pressurized, may be sprayed 10 minutes after take-off, keeping ventilation closed for 5 minutes. Cabins pressurized during flight may be sprayed before take-off, just after closing doors and windows. Military aircraft may be sprayed 5 minutes before starting the engines. In some cases spraying on arrival is substituted for or

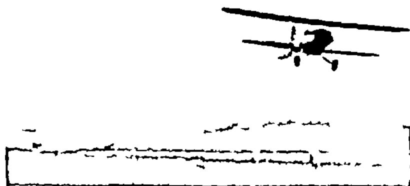


FIG. 59. Area distribution of DDT from an aeroplane. Tennessee Valley Authority, July–August 1943. (Courtesy TVA.)

added to the departure treatment. Cabins are done as soon as the engines have stopped and the rest of the plane is sprayed later when

the engines have cooled. Aircraft are kept closed for 5 minutes after spraying.

Some officials advocate combined aerosol and residual spraying of aircraft. Another point is that built-in spraying systems are effective and are becoming more common.

To guard against implanting exotic mosquitoes it is necessary not only to spray aeroplanes and, in some cases, other conveyances, but also to do away with potential breeding places in and around sea- and airports to make them inhospitable to mosquitoes.

Drainage and Filling

In many areas in the past, drainage has been unquestionably the best measure of malaria control. Even now, with all the new insecticides, there are still malaria control problems best solved by *immediate drainage*. There are other areas in which residual sprays make possible relief from malaria while plans can be made and

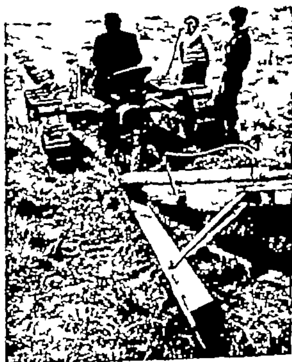


Fig. 60. Martin ditcher pulled by small tractor being used in Sardinia.

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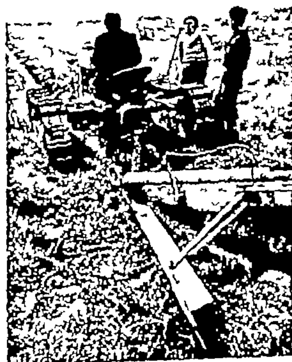


FIG. 60 Martin ditcher pulled by small tractor being used in Sardinia.

money secured for the more permanent benefits of drainage. A recent tendency has been to overlook drainage, but it becomes

increasingly obvious that there are no chemical panaceas. Proble[ms] vary and their solutions will also be diverse. One tries to accompli[sh] malaria control in the most logical way, all local factors considere[d].

No drainage project should be undertaken without careful pre-liminary planning and any scheme more complicated than simple short ditching requires technical knowledge and experience not possessed by the average health officer. Expert assistance should be requested from the engineering profession because drainage requires that careful levels be run, that intelligent plans be made as to relation of the proposed drainage system to other sanitary improvements, to existing water supplies, to disposal of sewage, and to maximum amount of water to be handled in the rainiest season.

Drainage may be accomplished in a great variety of ways, such as by surface ditches, sub-surface drains, vertical drains, pumps, tide gates and other devices. In every case the primary objective is to drain so effectively that regardless of weather or tide there will be no mosquito breeding, and to do the work in such a way that it enhances the value of the area affected. Just what type of drain is best for a given area will depend on topography, soil, climate and rainfall, as well as on economic factors. Cost will vary greatly depending on local factors, but [illegible] drains [illegible] drain they are too expensive even if they cost very little.

Surface drains may be simple [illegible] elaborate concrete channels [illegible] considerable amount [illegible] that drains be of solid construction [illegible]. When any few and as small as is compatible with the [illegible] ditches should be as clean-cut, sloping edges with narrow bottom and straight courses, avoiding sharp bends; they should have free flow of water at all times. All ditches require maintenance to insure [illegible]. Ditches may be dug by hand, by ditching machinery such as ploughs or excavators and drag lines, or by dynamite.

In contrast to surface drainage there is under-drainage by which water is conveyed beneath the surface of the ground. Nature's method of under-drainage is a layer of gravel or sand situated below the upper soil layer. Water readily passes through gravel or sand to some point of discharge. However, in many places the layers of the ground are so arranged that water is held in the upper soil producing swampy or wet areas not suitable for agriculture, but ideal for mosquitoes. In such areas subsoil drainage aims to remove the excess soil and ground water. In effect this under-dra[inage]

reduces the height of the water table. Subsoil drains are also useful for intercepting seepage water outcrops and have also sometimes been used to put a small stream entirely underground

Just as in surface drainage schemes, so in under-drainage it is vital to the success of the project that preliminary studies be made. Test borings will reveal sub-surface configurations and permit a logical plan to be drawn. There should be blueprints or diagrams not only of surface contours but also of the sub-surface strata to a depth somewhat below that of the proposed drain.

Subsoil drains may consist simply of gravel or rubble, or they may be made of tile or other pipes laid end to end with open joints. It is usual to begin at the outlet and to proceed up grade it is important to lay pipes deep enough to avoid clogging by grass and other roots and it is essential that careful levels be maintained. A final map is required, with pipe locations marked in such a way that a blocked pipeline could be dug up if necessary some years later Inspection chambers or wells are usually provided at intervals along the pipeline.

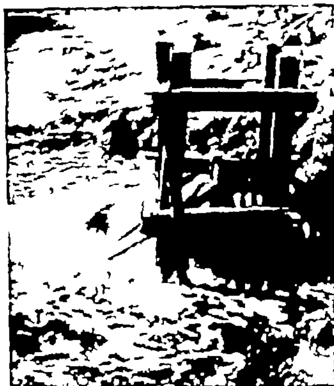
In Malaya, water from anti-malaria subsoil drains has been cleverly utilized for wells, washing-places and in one case for a swimming pool. Such thoughtfulness sweetens relations between health officers and laymen, adding little to the cost of the control project.

In limestone regions it may be possible to drain a pond or lime sink by boring a vertical drain through an impervious stratum to an underground channel. All the water will drop out through the vertical tunnel and will flow off underground in a natural water-course.

Drainage of tidal lands requires not only ditches but also bunds or embankments to keep out the sea at high tide. The drains discharge at low tide through automatic tide gates which close as the sea comes against them, but open again as the tide recedes, thus allowing the drains to empty Many types of tide gates have been described, the simplest one being a wooden box, set at the end of the drain and having a hinged flap on the sea side. All tide gates require supervision, because sticks and other debris frequently become impacted in such a way as to keep the flaps from closing

In some places it is economical to build solid embankments without gates and then pump the water out of the drains into the sea. Where land is very low and yet is so situated that it can be

utilized for agricultural purposes, as for sugar-cane, pump drainage is a useful procedure.



[illegible] Control. The Commonwealth Fund.

Filling. Sometimes mosquito breeding places can be eliminated by filling. Rock and tree holes can be filled with earth, sand, asphalt, or cement; rivers can be made to deposit silt in low areas (the French have given the word *colmatage* to such silting); mud can be raised by hydraulic pumps and dredges; and there are all sorts of dry fills making use of earth, gravel, rubbish, and waste materials such as cinders, ashes, and sawdust. Modern dirt moving equipment with bulldozers, power graders, power shovels, and drag lines have simplified certain types of filling operations. But large fills for mosquito control should not be advocated until it is clear that drainage will not dry an area. Finally, sometimes simple grading is effective.

Water management. Excellent water management for malaria

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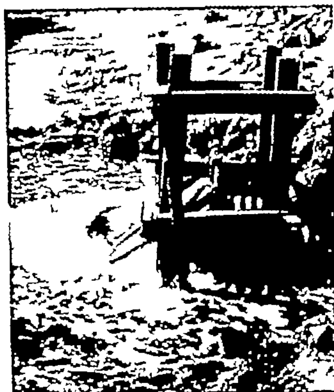


Fig. 61 Tide gate in use (Courtesy Herms and Gray, Mosquito Control, The Commonwealth Fund.)

Filling Sometimes mosquito breeding places can be eliminated by filling Rock and tree holes can be filled with earth sand, asphalt or cement rivers can be made to deposit silt in low areas (the French have given the word *colmatage* to such silting) mud can be raised by hydraulic pumps and dredges and there are all sorts of dry fills making use of earth, gravel, rubbish and waste materials such as cinders, ashes and sawdust. Modern dirt moving equipment, with bulldozers power graders power shovels, and drag-lines, have simplified certain types of filling operations. But large fills for mosquito control should not be advocated until it is clear that drainage will not dry an area. Finally sometimes simple grading is effective

Water management Excellent water management for malaria

control has been carried out by the Tennessee Valley Authority The malaria vector of the area is *A quadrimaculatus* whose preferential larval habitat is quiet, warm water with abundant vegetation Consequently the malaria problem areas in the Valley have been on the main river reservoirs and surrounding areas

Control was begun with an attempt *to build malaria out* while the dams were under construction. Past experience in the south-eastern United States had shown that impounding water in uncleared areas results in heavy *quadrimaculatus* output. So all trees, bushes and vines were removed from the plotted *recession zone* to provide a clean shore line Just before impoundage, the *water fluctuation zone* planned for malaria control was thoroughly cleared to insure a maximum of clean water surface for the first season. *Marginal* drainage ditches were dug to connect with the main body of the reservoir ponds which otherwise would be isolated.

Permanent shore-line improvements were made to reduce the costs of malaria control. There were *cut and fill* projects in which shallow areas, which would create favourable larval habitats, were deepened, and the earth removed was used to fill the remainder of the area, providing a new shore-line, abrupt, straight, and clean. The reclaimed land became valuable for agricultural and timber production There were also *diking and de-watering projects* in which shallow areas were diked off from the main reservoir Pumping stations were built into the dikes and water was pumped out of the shallow area during the mosquito season but allowed to flow back to provide food and shelter for waterfowl at other seasons.

In some areas, by purchase of *easement rights* the land was restricted to daytime use, all persons being sent out of the area during the hours when *quadrimaculatus* feeds.

The water-level management for malaria control each season involves various combinations of *flood surcharge constant pooling cyclical fluctuations* and *seasonal recessions* In the late fall, winter and early spring the water levels rise above normal needs so that, when the normal level is established, the drift and floatage are stranded on the banks above the fluctuation zone. During the spring growing season the water level is kept constant at such a level that the growth of vegetation in the fluctuation zone is retarded. When mosquito breeding starts a fluctuation schedule is established to provide regular lowering and raising of water levels to destroy mosquito larvae and pupae and to retard vegetation. Seasonal recession of

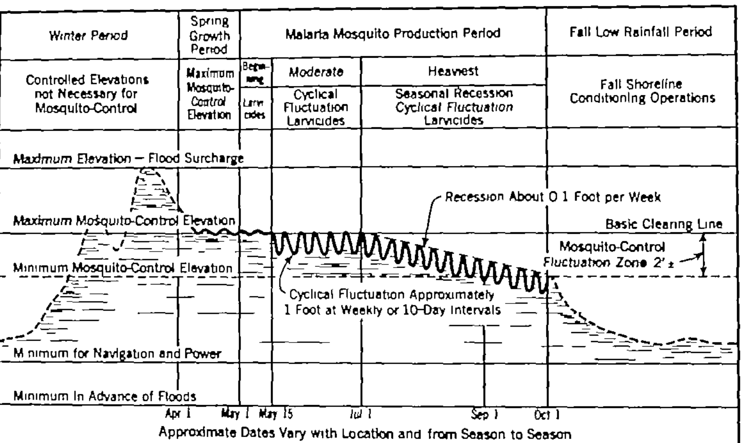


Fig. 62. Tennessee Valley Reservoir Malaria Control. (Courtesy TVA.)

level is begun when heavy mosquito production starts in early summer

In some areas such herbicidal sprays as 2,4-D have been used to control growth of vegetation liable to shelter larvae. Some aeroplane distribution of DDT larvicides and some annual removal of seasonal growth from the recession zones have been carried out, as well as some mosquito-proofing of homes in certain areas. There have also been some premises-spraying of residual DDT and, at all times careful surveys of malaria incidence and vector density

Sluicing or flushing Mosquito larvae and pupae breeding in small streams may sometimes be destroyed by periodically discharging into the stream enough water to flush out its edges. This is usually accomplished by alternately damming back water and then releasing it by hand removal of sluice gates or by automatic siphons The method is successful if there is an abundant supply and sufficient head of water with ample gradient to maintain the sluice wave and effective onrush of water Side pools and seepages require special attention

Intermittent drying The intermittent drying of irrigated fields and field channels is sometimes a practical measure of mosquito control. Depending on the local climate and soil, a suitable cycle of wet and dry periods is arranged so that the surface water and film on the fields is absent for a few hours once each week or 10 days thus permitting the aquatic stages of mosquitoes to be killed by the sun The dry period should not be long enough to permit the subsoil to become dry or to allow cracking of the soil or clod formation. Observing these precautions, one may expect normal or even better crops from an intermittently irrigated field. This measure requires sluice gates which can be tightly closed, field channels properly arranged, competent supervision and close co-operation by those cultivating the fields.

Other measures *Flooding* in some situations will control mosquito breeding by lessening the breeding area, raising the edge so that fishes and predators can get at the mosquito larvae increasing silt, and washing away the aquatic stages. Sometimes by *salinification* or by *sweetening* through suitable use of dams or diversion channels, a preferential larval habitat can be so altered as to discourage all breeding of a troublesome species of *Anopheles* *Diversion* of a residual flow in a dwindling river may obviate river bed pools. *Emptying* or *destroying containers* is an important control measure in

areas where aquatic stages of the vector or pest mosquito are found in such places as water jars, rain barrels, eaves gutters flower pots, old tins or tyres coconut shells, and cacao pods Copper sulphate will destroy bromeliads in which mosquitoes breed.

Naturalistic or biological control A naturalistic measure of mosquito control is one which extends or intensifies natural limiting factors without relying on chemicals or on mechanical devices. In other words, water manipulation drainage, and larviciding procedures are not naturalistic although sometimes they imitate natural control Under the classification of naturalistic control come such measures as exposing to sunlight or creating shade to turn away a shade-loving or a sun loving species from a preferred breeding place altering flora or fauna to increase the numbers of a plant growth like *Wolffia* or of fishes such as *Gambusia* polluting with waste material or rotting vegetation, drying by planting Such measures under special circumstances may be relatively cheap and effective.

Malaria vector eradication versus vector reduction No one now doubts that it is possible to eliminate all transmission of malaria in an area by reducing the numbers of the vector anopheline species until they are below a certain practical or *critical density* the magnitude of which depends on the natural history of mosquito and of man, as explained in earlier sections. But most vector control measures must be repetitive Hence the semantic appeal of the term *vector eradication* which suggests the ending of a problem by one complete effort and thus sounds more attractive than the term *vector reduction* Stimulated by the successful eradication of *A gambiae* from Brazil and from Egypt, some observers have come to advocate vector eradication as the method of choice to accomplish malaria control almost anywhere.

It is now becoming clear that only under special conditions is eradication the method of choice for malaria control. In most situations the elimination of a mosquito species is a costly and difficult undertaking which even if successful does in fact require never-ending maintenance measures On the other hand the elimination of malaria transmission by such a mosquito-reducing measure as residual spraying is simple and relatively inexpensive although to be sure it must be continued indefinitely The success of residual spraying in Greece Italy Venezuela British Guiana Tobago, Brazil Ceylon Bombay State Mauritius and elsewhere has been very convincing Moreover residual spraying

accomplishes more than malaria control. It is an effective way of dealing with flies fleas lice, and other household insects and, increasingly it is being demanded by householders whether malaria is a problem or not. So that even if a malaria vector has been eradicated, there will still be need in most communities to do residual spraying as well as to maintain a quarantine and scouting service to guard against reappearance of the malaria vector

On examination, it is seen that the eradication of *gambiae* from Brazil and Egypt did not eradicate malaria from the areas and did not prove that eradication is necessarily the best malaria control method somewhere else. For *gambiae* was a foreign invading mosquito with specialized larval habitats and adult behaviour patterns which made it highly susceptible to attack. Shannon who discovered it in Brazil, where probably only a single strain was present, wrote Owing to the unique conditions of the country and certain biological peculiarities of *gambiae* I was able in three months time to map the infested area and also to reach the con clusion that the species could not only be controlled but even exterminated in the territory it had succeeded in invading In both Brazil and in Egypt not only was a limited, sharply focused attack sufficient to deal with *gambiae* but there was present in each country a tremendous sense of disaster so that emergency measures could be readily imposed on the people. However it should be noted that Shannon s observations in Brazil were disregarded for more than 5 years, so that the infestation had time to become well established.

Anopheles eradication in places like Sardinia and Cyprus is a different matter For example, a species like *labranchiae* in Sardinia is an old-time resident, arousing no sense of impending doom *Labranchiae* is eclectic in its habits to such a degree that almost all water collections on the island are potential larval habitats and must be scouted and larvicided in an eradication project. Only limited larviciding of water surfaces was required in Brazil and Egypt, with practically no clearing or draining involved But in Sardinia a great deal of expensive clearing and draining was required simply to get at *labranchiae*—to find it and to kill it. When the Sardinian project ended in October of 1950 it had cost over 12 million dollars and had not eradicated *labranchiae* Both in Sardinia and in Cyprus it must be presumed that there remain foci of the anophelines which were under attack. Maintenance and quarantine must go on in definitely Sardinians already demand continued residual spraying although malaria is now uncommon.

While the Sardinian project can be justified as a useful way of applying post war UNRRA and ECA funds and specially as an experiment to reveal the basic difficulties of eradication and while it certainly has lifted a great burden of malaria from the people and in this way and also by drainage has opened large areas to cultivation it cannot be justified on the basis of malaria control alone. As an experiment it demonstrated how *not* to control malaria in such an area There is every reason to believe that had residual spraying been carried out on Sardinia as it was on the Italian mainland malaria would have been as successfully subdued on the island as it has been on the mainland. In other words the capital expenditure on Sardinia has not accomplished more in this respect than was done on the mainland nor has it obviated the need for continued house spraying Such spraying under Italian conditions costs less than 50 cents *per capita* per year or a total of about $2 50 *per capita* for the 5 years, 1946–50 On Sardinia, this cost must be contrasted with the $10 to $11 50 *per capita* cost of the eradication project.

Eradication costs in Cyprus have been less, and it may be that eradication was the best method on that island But there the householders have not had the concomitant benefits of residual spraying and drainage. Moreover the limited scouting employed on Cyprus makes it uncertain that eradication has been accomplished although malaria has been eliminated. With the maintenance service now in operation, the island should remain non malarious Neither in Cyprus nor in Sardinia can it be said that the costs have been out of proportion to the benefits derived by the people Moreover the lessons which have come from the two experiments have great value It is now apparent that an area chosen for an eradication project should not contain any major economically unmanageable tracts, that careful surveys should precede and accompany eradication work, that no preconceived rules should be allowed to restrict the adoption of new and promising techniques in practice and that no special preference should be shown for either anti adult or anti-larval measures.

Eradication is undoubtedly a method which may be applicable to special areas. For instance perhaps it is the method of choice against *pseudopunctipennis* in certain isolated Pacific slope villages in Peru and Chile or against the invaders *gambiae* and *funestus* on Mauritius Elsewhere as in Ceylon against *culicifacies* it would probably be too costly a measure to be feasible In other words it is still true that no method of malaria control is universally applicable. One must

always attempt to achieve the goal in the most logical fashion. If speed is required, then more money will be needed if the vector has sharply delimited larval habitats, it may be vulnerable to eradication. But if eradication is decided upon both time and money must be available without limit, even if it costs $100 000 to eradicate the last 10 individuals of the species under attack. Had there been sufficient funds available in Sardinia in 1949 and 1950 to repeat the 1948 programme several observers believe that eradication of all anophelines, excepting the tree-hole *plumbeus* would have been achieved.

Control by attacking the plasmodium Destroying the causative agent might seem logically to be the most direct way of controlling malaria in a community but up to the present time this line of attack has not had much success. In the first place, there are no practical methods for producing artificial immunization against malaria by use of sera and vaccines secondly the use of prophylactic drugs has not yet become a thoroughly practical measure for communities although the addition of chloroquine and chlorguanide to the armamentarium has increased the chances of success.

As already noted, individuals desiring to protect themselves from acute malaria while exposed to infection may use quinacrine, chlorguanide, chloroquine, or camoquine in the doses given and with the limitations and the results already described These drugs afford a high degree of protection from clinical malaria but frequently do not prevent relapses of vivax malaria. These drugs are also useful in protecting groups of soldiers, labourers or others who must live and work in malarious places which cannot be made safe by anti-mosquito measures.

But for the routine control of malaria in a community it is not yet possible for practical reasons to recommend mass suppressive therapy Anyone who has ever attempted to administer any sort of medication to every individual in a town regularly week by week, knows how difficult are the problems of absentees, infants and toddlers, and assorted individualists. Skilled personnel are required to administer the drugs which may not be left with the householders or at dispensaries in this type of malaria control. Thus costs are higher as a rule, often considerably higher than required for residual spraying or larviciding Nevertheless with the newer drugs there may now be communities in which it will be cheaper to use prophylactic drugs than to destroy the mosquito vector

Only careful trials will give an answer to this question Certainly the outstanding success of residual spraying projects at low cost in many parts of the world must not be forgotten when one is considering the use of suppressive therapy on a community basis

Maintenance Sometimes when a control programme has driven malaria incidence down to a low point, there is a tendency for responsible officials to consider the problem permanently solved and to make no further provision for preventive measures This of course, is a mistake especially in the tropics Having stopped malaria transmission it is necessary to carry out *maintenance* or what has been called *strategic control* in order that malaria may not reappear This protects the investment and can be done at relatively low cost. Usually a restricted or modified spraying programme is continued with special attention to other household insects Whether residual applications will be required every year or less frequently is a question to be considered carefully Full use should be made of natural barriers and any other natural controlling factors. Permanent environmental improvements, perhaps not feasible previously should also be considered.

Some economic considerations Surveys to determine what it is costing a community to submit to malaria, or to other mosquito-borne disease may help to arouse local desires for a control project. The following items should be kept in mind

I *Deaths from mosquito-borne diseases*

1 Value of a life lost.

2. Cost of funerals of those who might have lived long enough to earn this cost.

II *Illness from mosquito-borne diseases*

1 Cost of medical care (physicians quacks, medicines)

2. Cost of spiritual care (priests, quacks candles, sacrificial animals and offerings)

3 Lost earnings

(*a*) Directly due to illness (*b*) due to nursing a relative or friend (*c*) for interest on borrowed money

4 Lessened efficiency of convalescents and chronic cases

(*a*) Lower output of labour resulting in smaller crops lessened manufacturing (*b*) lack of mental and physical energy leading to apathy stagnation of a community or even regression.

III. *Material losses due to mosquitoes*

1. Lower rentals.
2. Depreciated real estate.
3. Forced sales.
4. Loss of sleep and working time.
5. Loss and depreciation of livestock.

Residual spraying with good control of malaria in a community has been carried on at costs varying in different countries from less than 10 cents to more than $2 *per capita* per year (U S currency) In India excellent results have been reported with DDT residual spraying For example, in the Bombay State project, 2 million homes are being sprayed thus protecting 9 million persons at a cost of 2·7 million rupees. This is about 6 3 U S cents *per capita*—surely a first-class investment! In Italy on the mainland, malaria has been almost eradicated by residual spraying alone, and the costs have not exceeded 50 cents *per capita* per year in fact, have been about 35 cents in several areas. Such examples indicate that it is no longer logical to delay malaria control because of the cost, for the facts now indicate that no country however under-developed can afford not to control malaria by modern, cheap and effective methods



FIG. 63 A multiple family house abandoned because of the malariousness of the area.

Recommendations to governments The World Health Organization is doing much to prove to governments the economic

feasibility of malaria control to demonstrate the indirect benefits accruing in general public health and welfare and in increased agricultural and industrial production and to assist and encourage governments to work towards nation wide control and eradication of malaria by well-organized routine application of modern methods. The following statement was distributed in 1950 to all countries where the disease is a problem This memorandum so clearly expounds the subject that it is reprinted here

1 The World Health Organization desires to stress the importance of malaria, which still attacks hundreds of millions of persons each year causing millions of deaths Malaria is a major source of mental apathy and general physical deterioration and this is responsible for a serious loss of working efficiency wherever the disease is endemic Malaria is a direct factor in the world shortage of food and it causes grave interference with industrial and agricultural development and enterprise The WHO urges each government faced with a malaria problem to attack it as promptly and vigorously as possible

2 The WHO also desires to emphasize that to-day at financially feasible costs, governments can attain a degree of practical malaria control, and even malaria eradication utterly impossible fifteen years ago

3 Further the WHO desires to make the following particular points about malaria control, based on deliberations of the Expert Committee on Malaria of the WHO.IC and of the WHO at sessions held in 1947 1948 and 1949 and on documents and resolutions of the First and Second World Health Assemblies in Geneva in 1948 and in Rome in 1949 respectively

(i) The first essential for effective control of malaria in any country is the establishment on a permanent basis of a malaria control organization of adequate size staffed by adequately paid and adequately trained personnel.

(ii) In the existing state of knowledge measures directed against the mosquito transmission of malaria are the only methods which give effective control. They should take priority wherever possible

(iii) The application of a modern insecticide such as DDT as a residual spray on the inside walls of habitations and other mosquito shelters to kill adult anopheline vectors is the most effective method of mass attack on malaria in rural areas so far evolved. The practical success of residual spraying already demonstrated in many parts of the world has advanced this method well beyond the experimental stage and it can now form a basis for extensive malaria control programmes However it is essential that spraying operations be organized systematically keeping in view such factors as natural history of the local

vector species, nature of sprayed surfaces, formulation and dosage of insecticide frequency of spraying and selection of suitable equipment.

(iv) Chemotherapy specially with the newer antimalarial drugs, is essential in the clinical control of *epidemics* of malaria, but it is clear that chemotherapy plays a secondary role in the prevention of malaria. While therapeutic and prophylactic antimalarial drugs should be available to those who require them (regardless of ability to pay for such treatment) it must be emphasized strongly that in numerous rural areas throughout the world the use of residual sprays has in two or three seasons made mass chemotherapy and mass chemoprophylaxis of malaria unnecessary and obsolete Contrariwise, intensive distribution of free antimalarial drugs for many years in other areas has had no marked influence on the incidence of malaria, except in well-supervised groups.

(v) Methods of irrigation cultivation, and animal husbandry should be improved where necessary so that they will tend to reduce rather than intensify malaria prevalence. In particular new public works, including irrigation and road construction, should be carefully planned in consultation with the malariologist so that they will not become, as has frequently happened in the past, a source of malaria.

(vi) Sometimes much malaria can be averted in housing projects if they are so planned that malarious sites are avoided and malariogenic conditions are not created by construction operations.

(vii) Governments should actively support scientific research directed specially towards improving malaria control and therapy

(viii) Legislative enactments are essential to give legal sanction and support to appropriate malaria control measures within a country

(ix) The prevention of the exportation and importation of living anophelines (as well as other arthropods) is in many countries a matter of great importance and requires the co-operation of all governments in carrying out suitable quarantine measures under national and inter national regulations Further it is urged that all countries confronted with the problem of malaria should take active measures to prevent the spread of anophelines within their own borders.

(x) While it is recognized that a reasonable amount of malaria survey work should precede control, yet it must be emphasized strongly that where malaria is known to be a problem it is important to start residual spraying at least as a pilot experiment, as soon as possible and not delay for the purpose of extensive studies. Concurrent entomological and epidemiological investigations are essential for checking and for guidance. But case finding, and tracing of carriers—according to present experience—soon lose significance when active residual spraying is being carried out. Notification of malaria cases, of course, is still of importance to a public health department.

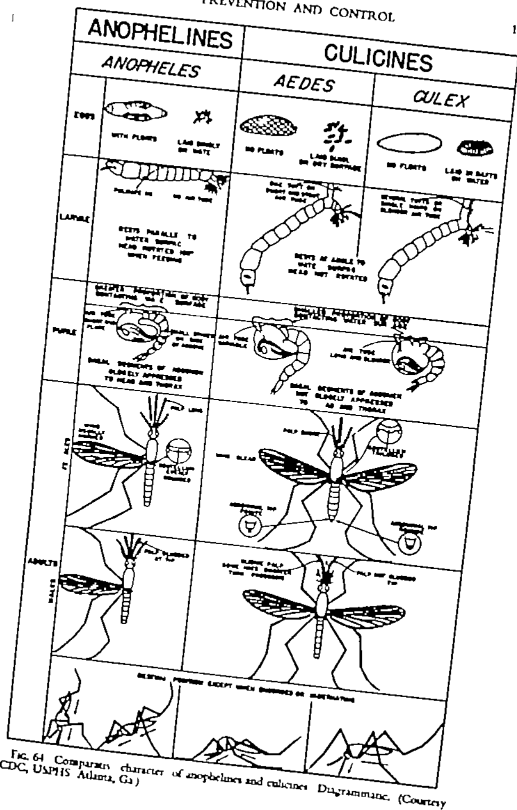


FIG. 64 Comparative character of anophelines and culicines. Diagrammatic. (Courtesy CDC, USPHS Atlanta, Ga.)

4 Finally the WHO calls attention to the fact that, within limitations of its budget and through its Regional Organizations, it is prepared to give on request certain types of malaria control assistance to governments and help to institutes devoted to training in malariology In particular it will make available (*a*) expert advice and technical reports on the control of malaria and on the education of the public in malaria prophylaxis (*b*) fellowships and travel grants for malaria control training (*c*) teams and individual experts to carry out demonstration malaria control programmes and to develop malaria control organizations (*d*) lecturers, literature, and teaching equipment to schools of malariology

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MEASUREMENTS AND EQUIVALENTS

LENGTH

1 inch (U S) = 2·54001 cm = 25·4001 mm. (British inch = 2·539998 cm.)
12 in = 1 foot = 0·304801 m.
3 ft. = 1 yard = 0·914402 m
5½ yds = 1 rod = 25 links = 5·02921 m.
320 rods = 1 mile (5,280 ft. or 1 760 yds) = 1·60935 km. = 1 609·35 m.
1 knot or nautical mile = 1·1516 statute miles = 6,080·27 ft. = 1·85325 km.
1 millimetre = 0·001 m. = 0·03937 in.
10 mm = 0·01 m. = 1 centimetre = 0·3937 in
10 cm. = 0·1 m. = 1 decimetre = 3·937 in.
10 dm = 1 metre = 39·3700 in. (British, 39·3701 in) = 3·280833 ft.
10 m = 1 dekametre = 10·9361 yds
10 dkm. = 1 hectometre = 19·8838 rods.
10 hm. = 1 kilometre = 0·621372 mile = 1 093·6 yds. = 3,280·8 ft.
1 micron (μ) = 10^{-4} cm = 0·001 mm. = 3·937 × 10^{-5} in
1 link = 7·92 in = 20·11684 cm
100 links = 1 chain = 66 ft. = 22 yds. = 4 rods = 20·11684 m.
1 furlong = 40 rods = 220 yds = 201·1684 m.
1 hand = 4 in = 10·160 cm

SIEVE MESH SIZES IN MICRONS

Sieve Number	*Screen Mesh Size (microns)*
10	2,000
20	840
50	297
100	149
120	125
140	105
170	88
200	74
230	62
270	53
325	44
400	37

MASS

1 microgram (μg or γ) = 0·001 mg
1 milligram = 0·015432356 grain
10 mg = 1 centigram = 0 15432356 grain
10 cg = 1 decigram = 1 5432356 grains.
10 dg = 1 gramme = 15·432356 grains.
10 gm = 1 dekagram = 5 643833 drams avdp
10 dkg = 1 hectogram = 3 527396 oz. avdp
10 hg = 1 kilogram = 2 2046223 lb avdp

1 gramme = 0·00220462 lb avdp = 0·00267923 lb apothecary or troy
1 gramme = 0·0352739 oz. avdp = 0·0321507 oz. apoth or troy
1 gramme = 15·4324 grains.
1 kg = 35 273957 oz. avdp = 32 150742 oz apoth or troy
1 kg = 2 2046223 lb avdp = 2 6792285 lb apoth. or troy

27 34375 grains = 1 dram avdp = 1 771845 gm
16 drams = 1 ounce = 28 349527 gm
16 oz. = 1 pound = 453 5924 gm = 0 4535924 kg
2,000 lb = 1 ton (short) = 907 18486 kg
1 long ton (U S or British) = 1 120 short tons = 2,240 lb avdp
1 metric ton (*tonne*) = 2,204·62 lb avdp = 1 000 kg
1 metric ton = 0·984206 long ton = 1 10231 short tons
1 British stone = 14 lb avdp = 6 35 kg
1 grain avdp., apoth or troy = 64 798918 mg
20 grains = 1 scruple = 1 2959784 gm.
3 scruples = 1 dram apoth. or troy = 3 8879351 gm
8 drams = 1 ounce apoth or troy = 31 103481 gm.
12 ounces = 1 pound apoth or troy = 373 24177 gm

AREA

1 square millimetre = 0·00155 sq in.
100 mm^2 = 1 sq centimetre = 0 155 sq in.
100 $cm.^2$ = 1 sq decimetre = 15 5 sq in
100 dm^2 = 1 sq metre = 10·76387 sq ft. = 1,550·0 sq in.
1 sq metre = 1 195985 sq yds (U S) = 1 195992 sq yds. (British)
10 000 sq metres = 1 hectare = 2·471044 acres (U S)
10 hectares = 1 sq kilometre = 0 3861006 sq mile (U.S)
10 sq chains = 1 acre = 43,560 sq ft = 4 840 sq yds. (U S)
1 acre (U S) = 4 046 873 sq metres.
1 acre (British) = 4 046 849 sq metres
1 sq mile = 640 acres = 2 589998 sq km

VOLUME

1 cu. mm = 0·0000610234 cu. in.
1 000 mm.^{3} = 1 cu cm = 0·0610234 cu. in.
1 000 cm.^{3} = 1 cu dm. = 61·0234 cu. in
1 000 dm ^{3} = 1 cu. m = 1 3079428 cu yds (U S) = 1 307954 cu yds. (British)
1 m.^{3} = 35 314445 cu. ft. (U S) = 35 31477 cu. ft. (British)
1 cu. in. (U.S) = 16 387162 cm ^{3}
1 cu. in. (British) = 16 3870253 cm.^{3}
1 728 cu in. = 1 cu ft. = 0·0283170 m.^{3} (U S)
1 cu. ft. (British) = 0 02831677 m ^{3}
27 cu. ft. = 1 cu. yd. = 0 76455945 m.^{3} (U S)
1 cu. yd. (British) = 0 76455285 m.^{3}

CAPACITY

1 millilitre = 16 2311 minims = 0 0084538 gill (U S)
1 ml. = 0·0338147 oz. (fluid, U S) = 0·035196 oz. (fluid, British)
1 ml. = 0·061025 cubic inch = 1·000027 cubic centimetres.
10 ml. = 1 centilitre 10 cl. = 1 decilitre 10 dl. = 1 litre.
1 l. = 1 000 ml. = 1 056710 quarts (liquid, U S) = 0 264178 gal. (liquid, U.S)
1 l. = 0 26417762 gal. (U.S) = 0 21998 gal (British)
1 l = 0·028378 bushel (U S) = 0·027497 bushel (British)
1 l. = 33 8147 ounces (fluid, U S) = 35 196 ounces (fluid, British)
4 gills = 1 pint 2 pts. = 1 qt. 4 qts. = 1 gallon.
1 pint (liquid, U S) = 473 179 cubic centimetres = 473 167 millilitres.
1 pint (liquid, British) = 568 26 cubic centimetres = 568 25 millilitres.
1 quart (liquid, U S) = 0 946333 litre.
1 quart (liquid, British) = 1 13650 litres
1 gal. (U S) = 231·00 cubic inches = 3 7853 litres.
1 gal. (British Imperial) = 277 3 cubic inches = 4 54596 litres.
1 U S. gal. of water at 15 C. (62° F) weighs 3 7820 kg or 8 337 lb (avdp)
1 Brit. Imperial gal. of water at 15 C (62° F) weighs 4 535924 kg or 10 lb (avdp)
1 Brit. Imp gal. = 1 20094 U S gals.
1 U S gal. = 0 83268 Brit. Imp gals.
1 U S liquid barrel = 31 5 U.S gals.

APOTHECARIES FLUID MEASURE

60 minims = 1 fluid dram = 3·6966 millilitres.
8 fl. dr = 1 fluid ounce = 29·5729 millilitres.
16 fl. oz. = 1 pint = 0·473167 litre
1 U.S. minim = 0·061610 millilitre = 0·061612 cubic centimetre
1 British minim = 0·059194 cubic centimetre

TEMPERATURE CONVERSION TABLE

C.	F.	C.	F.
− 34·4	− 30·0	15·6	60·0
− 30·0	− 22·0	20·0	68·0
− 28·9	− 20·0	21·1	70·0
− 23·3	− 10·0	23·9	75·0
− 20·0	− 4·0	25·0	77·0
− 17·8	0·0	26·7	80·0
− 12·2	10·0	30·0	86·0
− 10·0	14·0	32·2	90·0
− 6·7	20·0	35·0	95·0
− 1·1	30·0	37·0	98·6
0·0	32·0	37·8	100·0
1·7	35·0	40·0	104·0
4·4	40·0	40·6	105·0
5·0	41·0	43·3	110·0
7·2	45·0	45·0	113·0
10·0	50·0	46·1	115·0
12·8	55·0	48·9	120·0
15·0	59·0	50·0	122·0

1 C. = 1·8 F.
1 F. = 0·5556 C.
$\frac{9}{5}$ C. + 32 = F.
$\frac{5}{9}$ (F. − 32) = C.

GENERAL INDEX

INDEX OF PLACES

INDEX OF PERSONS